Prevalence of *Escherichia coli* Serotype O157:H7 in Four Species of Apparently Healthy Domestic Animals

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Dedication

To my father, mother

sisters and brother

Manar

Preface

This work was carried out at the Department of Preventive Medicine and Public Health, Faculty of Veterinary Medicine, University of Khartoum, under the supervision of Dr Abdelwahid Saeed Ali.

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Abstract

Escherichia coli serotype O157:H7 is the most common shiga-like toxin producing *E. coli*. It has been associated clinically with severe bloody diarrhea or hemorrhagic colitis, which can lead to hemolytic uraemic syndrome (HUS).

This study was carried out to determine the prevalence of *E. coli* O157:H7 in different domestic animal species.

Forty five fecal samples and 60 rectal swabs from apparently healthy domestic animals were collected from Shambat, Alkalakla and Kuku localities in Khartoum State.

All samples were cultured on sorbitol MacConkey's agar and MacConkey's agar, isolates from the fecal samples and rectal swabs were identified by their microscopic features, biochemical properties and antibiotic sensitivity test.

E. coli O157:H7 was isolated from 8 fecal samples (17.7%), most frequently from sheep (20%) and goats (20%) and less frequently in cattle (13.3%).

E. coli O15:H7 was isolated also from 5 rectal swabs (8.3%), most frequently from goats (13.3%) and less frequently from sheep (6.6%), cattle (6.6%), and chickens (6.6%).

In total, out of 105 samples, a prevalence of 12.3% (13 isolates) of *E. coli* serotype O157:H7 was detected.

All *E. coli* isolates from fecal samples were subjected to antibiotic sensitivity test. The result showed resistance of all isolates to Ampicillin/sulbactam at 20 mcg concentrations, and high sensitivity to chloramphencol, gentamicin and pefloxacin, while the susceptibility to the other antibiotics was variable.

الخلاصة

الاشرشيا القولونية ذات النمط المصلى O157:H7 و التى تنتج سموما مشابهة لتلك المنتجه بواسطة البكتريا من جنس الشيقيلا ،تعتبر ذات صله اكلينيكية بالاسهال الدموى الحاد (hemorrhagic colitis) الذى قد يؤدى الى اصابة الكلى بمتزامنة البول الدموي (hemolytic uraemic syndrom) .

هدفت هذه الدراسة الى معرفة انتشار هذا النمط المصلى بين الحيوانات الاليفة (البقر، الضأن، المعز و الدواجن).

جمعت ٤٥ عينة براز و ٦٠ مسحة من المستقيم من حيوانات سليمة ظاهرياً من المناطق التاليه: شمبات، الكلاكلة وحلة كوكو بولاية الخرطوم.

زرعت كل العينات التي تم جمعها في أجار سوربيتول ماكونكي و أجار ماكونكي. التعرف على البكتريا المعزولة تم بواسطة الفحص المجهري، الخواص الكيموحيوية و حساسيتها للمضادات الحيوية.

تم عزل الاشرشيا القولونية ذات النمط المصلى O157:H7 من ٨ عينات من عينات البراز(١٧,٧%)، وكانت نسب عزل هذه البكتيريا كالاتي:- الضأن (٢٠%)، الماعز (٢٠%) و الابقار (١٣,٣%).

أيضا تم عزل الاشرشيا القولونية ذات النمط المصلى O157:H7 من٥عينات من مسحات المستقيم (٨,٣%) أكبرنسبة عزل لهذه البكتيريا كانت من الماعز (١٣,٣%) بينما نسب العزل من الابقار،الضأن و الدواجن أقل بواقع (٦,٦٦%) لكلٍ.

الاشرشيا القولونية التى تم عزلها من عينات البراز تم اخضاعها لفحص حساسيتها لاثني عشر نوعاً من المضادات الحيوية، كل العزلات أظهرت مقاومتها للامبسلين/ سالباكتام وحساسية عالية للكورامفنيكول، الجنتاميسين وبفلوكساسين.

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Introduction

Escherichia coli (*E.coli*) was discovered by Theodor Escherich, a German pediatrician and bacteriologist; in 1885 he isolated *E. coli* from new born babies*. E. coli* is one of the main species of bacteria that live in the lower intestines of mammals. It is classified under the family Enterobacteriaceae and the genus Escherichia.

Escherichia coli is possibly the most studied bacteria as well as it is one of the most important disease causing agents. The organism is a normal inhabitant of the lower part of the intestinal tract of all warm-blooded animals. Being a primary component of faeces, it is therefore one of the most ubiquitous bacteria on the surface of the earth. The bacterium is necessary for the proper digestion of food and are apart of the intestinal flora.

Escherichia coli like many other enterobacteria, contain numerous serotypes some of which are associated with certain infection in man and animals. *E. coli* can be the causative agent of several intestinal and extra-intestinal infections such as urinary tract infections, meningitis, peritonitis, mastitis, septicemia and pneumonia.

E. coli serotype 0157:H7 which was initially recognized in 1982 in the United State, has now emerged as an important enteric pathogen of considerable public health significance (Samuel *et al.,* 1988).

E. coli O157:H7 is an enterohemorrhagic strain of the bacterium *Escherichia coli* and a cause of food-borne illness (Karch, *et al*., 2005). In 1999 it was estimated that about 37, 000 cases of infection and 60 deaths occur each year in the United States alone as a result of *E. coli* O157:H7 (Center of Disease Control, 2006).

Escherichia coli serotype O157:H7, which produces shiga-like toxin, known as verocytotoxin, is a known cause of hemorrhagic colitis and hemolytic uremic syndrome in man (Wells *et al*., 1991).

Most human infections acquired from the consumption of food and water contaminated directly or indirectly with animal fecal material, consumption of raw milk, under-cooked ground beef and unpasteurized fruit

juices had been linked epidemiologically with several outbreaks of disease caused by *E. coli* O157:H7 (McDonough *et al*., 2000).

The aim of this study is to determine the frequency of *E. coli* O157:H7 in populations of healthy carrier domestic animals namely cattle, sheep, goats and chickens, using the microscopic, cultural and biochemical properties of this serotype. Also, to determine the antibiotic susceptibility of the obtained isolates.

Chapter One

Literature Review

1-1- Historical Background and Classification of *Escherichia coli*:-

The dual nature of *Escherichia coli* as pathogen and commensal has long intrigued microbiologists. When Escherich (1885) first studied the fecal flora of neonates, he did so to gain a better understanding of the pathogenesis of enteric infections. As a result he stated that " this species is now know to be closely related to the enteric Bacterium coli commune", which now bears his name as *Escherichia coli* and this species is know to be closely related to the enteric pathogens *Shigella* and *Salmonella.* The need to differentiate between commensal *E. coli* and bowel pathogens at first led to the development of the biochemical tests that become the basis of modern bacterial taxonomy, notably the taxonomy of *Enterobacteriaceae* in general and *E. coli* in particular. The *Enterobacteriaceae* have been refined by Farmer *el al*., (1985).the major contribution of Kauffmann (1947) was to establish the serology of *E. coli,* which permitted a greater understanding of the ecology of these organisms. Other methods of typing have also successfully been applied to *E. coli* including biotyping, the use of the antibiotic resistance patterns (resistotyping) and isoenzyme patterns. The clonal nature of *E. coli* has been specifically addressed in a number of ways (Sussman, 1997).

The species *Escherichia coli* belongs to the genus *Escherichia* in the family *Enterobacteriaceae*, according to the nine edition of Bergy's Manual of Determinative Bacteriology (Bergey and Holt, 1994) in which the family *Enterobacteriaceae* includes the following tribes:-

1- *Eschercheae.*
2- *Kelbsielleae.*
3- *Proteusae.*
4- *Yersinieae.*
5- *Erwineae.*

The tribe *Eschericheae* includes five genera:-

1- *Escherichia.*

2- Edwardsiella.

3- Citrobacter.

4- Salmonella.

5- Shigella.

The genus *Escherichia* includes the following species:-

1- *E.coli.*

2- *E. adecarboxylata.*

3- *E. fergusonii.*

4- *E. hermenii.*

5- *E. vulneris.*

Other classification according to the sequences of the ribosomal RNA (rRNA) includes:-

Domain: Bacteria.

Phylum: Protobacteria.

Class: Gammaprotobacteria.

Order: Enterobacteriales.

Family: Enterobacteriaceae.

Genus: *Escherichia.*

Species: *Escherichia coli*.

1-2- Definition:-

The Protobacteria is the major group that includes a wide variety of pathogens such as *Escherichia*, *Salmonella, Vibrio*, *Helicobacter*, and many other notable genera (Madigan and Martinko, 2005). The group is defined primarily in terms of ribosomal RNA (rRNA) sequences, and is named for the Greek god Proteus, who could change his shape, because of the great diversity of forms found in this group (Wikipedia, 2007). All Protobacteria are Gram-negative, with an outer membrane mainly composed of lipopoly saccharides. Many move about using flagella, but some are non-motile or rely on bacterial gliding. There is also a wide variety in the types of metabolism. Most members are facultatively or obligately anaerobic and heterotrophic, but there are numerous exceptions (Wikipedia, 2007). The Protobacteria are divided into five sections, referred to the Greek letters alpha through epsilon, again based on rRNA sequences. Gamma protobacteria comprised several

medically and scientifically important groups of bacteria such as Enterobacteriaceae (Wikipedia, 2007).

Enterobacteriaceae is a large family of bacteria including many of the more familiar pathogens, such as, Salmonella and *Escherichia coli*. Members of the Enterobacteriaceae are rod-shaped, and are typically 1-5 μm in length. Like other Protobacteria they are Gram-negative, facultative anaerobes, fermenting sugar to produce lactic acid and various other end products, they also reduce nitrate to nitrite. Unlike most similar bacteria Enterobacteriaceae generally lack cytochrome oxidase, although there are exceptions. Most have many flagella, but a few genera are non-motile. They are non-spore forming, and with exception for *Shigella dysenteriae*, they are catalase-positive. Most members of Enterobacteriaceae have peritrichous type 1 fimbriae involved in the adhesion of the bacterial cells to their host (MacFaddin, 1980).

The genus *Escherichia* is composed of several species, but only *E.coli* is the important pathogen of animals. This species, the major facultative gram-negative species comprising the normal flora of the gastrointestinal tract, may be the cause of septicemic disease in foals, calves, piglets, puppies and lambs (enterotoxigenic diarrhea in newborn farm animals) and of edema disease in big. It is also considered as an opportunistic pathogen in almost all animal species (e.g. in urinary tract diseases, abscesses, and pneumonia) (Hirsh and Yuanchang, 1999).

Escherichia coli and its relatives are known to microbiologists as enteric bacteria, because they live in the intestinal tract of human and other animals. *E. coli* in the family *Enterobacteriaceae*, which is made up of Gram-negative, non-spore-forming,rod-shaped bacteria that are often motile by means of flagella. The majority of strains grow well on the usual laboratory media in the presence or absence of oxygen, and metabolism can either be by respiration or fermentation (Todar, 2005). It grows well at 37˚C, D-glucose and other carbohydrates are catabolized with the production of acid and gas. Oxidase negative and catalase positive (Bergey and Holt, 1994).

Escherichia coli ferment lactose and possess a typical Gram-negative cell wall containing lipopolysaccharides (LPS). Approximately 170 different O antigens have been delineated and some of these are cross-reactive with *Shigella, Salmonella* and *Klebsiella* (Douglas, 2007).

Motile strains possess H (flagellar) antigens that can be used for epidemiological purposes. *E.coli* also possesses K (capsular) antigens similar to the Vi antigen of *Salmonella*. Enterotoxigenic strains may also display colonization factor antigens (CFA/1, CFA/2) (Douglas, 2007).

E. coli is the major facultatively anaerobic organism in the intestinal tract of most animal species it's usually the dominant organism recovered on aerobic culture of faeces. But pet birds appears to be an exception as *E. coli* are recovered from only a low percentage of healthy pet birds (Carlton, 2004).

1-3- Habitat, Reservoir and Transmission:-

The Enterobacteria are world wide in distribution. They are both potentially pathogenic and non pathogenic species. Many of the Enterobacteria are part of the normal flora of the intestinal tract. Some species are free-living occurring in soil and water (Carter, 1986). Many members of the family are a normal part of the gut flora of the humans and other animals, while other are found in water or soil, or are parasites on a variety of different animals and plants (MacFaddin, 1980).

Strains of *Escherichia coli* capable of producing disease reside in the lower gastrointestinal tract and are abundant in environments inhabited by animals (Hirsh and Yuanchang, 1999).

For most of the 20th century, *E. coli* has been used as the principal and indicator of pollution in both tropical and temperate countries. *E. coli* comprises about 1% of the total fecal bacterial flora of humans and most warm-blooded animals. Sewage is always likely to contain *E. coli* in relatively large numbers. In addition, *E. coli*, being a typical member of enteric bacterium is presumed to have survival characteristics very similar to those as used an indicator of microbiological contamination. As such an indicator organism, its value is significantly enhanced by the ease with which it can be detected and cultured (Toda, 2005).

The sterile intestinal tracts of new born animals quickly become contaminated with bacteria from the dam and the environment. *E. coli* rapidly becomes established in the intestine and remains as a part of the normal flora throughout the life of the animal. The concentration of *E. coli* is low in the upper small intestine but it increase progressively, with the maximum

concentration in the large intestine. The vast majority of *E. coli* in the normal flora are non pathogenic, but shiga-toxin-producing *E. coli* (STEC) in the normal flora of cattle and other ruminants may be highly pathogenic for humans (Carlton, 2004).

The transmission of *E. coli* is almost always by ingestion. Fomites are especially important, although some infections are endogenous (Carter,1986). The bacterium is ingested in food or water or obtained directly from other individuals handling infants. The human bowel is usually colonized within 40 hours of birth; *E. coli* can adhere to the mucus overlying the large intestine. Once established, an *E. coli* strain may persist for months or years. Resident strains shift over along period (week to months), and more rapidly after enteric infection or anti-microbial chemotherapy that perturbs the normal flora (Todar, 2005).

1-4- Characteristics of E. coli:-

1-4-1- Morphology and Staining Reactions:-

Escherichia coli is a Gram-negative, rod shaped organism, measuring 1.0 to 1.5 µm in width by 2.0 to 6.0 µm in length and it varies widely in morphology under different conditions. Rods are usually short, plump and some times rather long filament are seen. It can be motile by means of peritrichous flagella or it can be non motile. *E. coli* never forms spores, and capsular materials are present on some strains. It stains readily and evenly with ordinary stains, fimbriae are frequently present (Hagan and Timoney, 1988).

1-4-2- Cell Structure:-

E. coli possesses adhesive fimbriae and a cell wall that consists of an outer membrane containing lipopolysaccharides, a periplasmic space with a peptidoglycan layer, and an inner cytoplasmic membrane. Some strains are piliated and capable of accepting and transferring plasmid to and from other bacteria. Such property enables *E. coli* under unfavorable conditions to survive. Even though it has extremely simple cell structure, with only one chromosomal DNA and a plasmid, it can perform complicated metabolism to maintain its cell growth and division (Joan and John, 2007).

1-4-3- Cellular Composition and Virulent Factors:-

1- Capsular Polysaccharides (K- antigens):-

K-antigens are important for those microorganisms that come in contact with the products and cells of the host, such as invasive strains of *E. coli*. The capsular substances protect the outer membrane from the complement cascade and inhibit the microbe from attachment to, and ingestion by phagocytic host cells. There are at least 80 distinct K-antigens in *E. coli* (Hirsh and Yuanchang, 1999).

2- Lipopolysaccharides (LPS):-

LPS is found in the cell wall, it's an important virulence determinant. There are approximately 165 serologically distinct O-groups (Hirsh and Yuanchang, 1999).

3- Flagellar (H-antigens):-

Almost all strains of *E. coli* are motile by means of peritrichous flagella; there are at least 50 flagellar antigens.

The O, H and K- antigens are used in serotyping a particular isolate (Hirsh and Yuanchang, 1999).

4- Adhesins:-

They mediate adherence to target cell in the gastrointestinal tract and to cells comprising the niche for the strain. Adhesins are important virulence factors only when the microbe is on mucosal surface (Hirsh and Yuanchang, 1999).

1-5- Isolation and cultural characteristics:-

E. coli grows readily on all ordinary media, the optimum temperature for growth is 37˚C but it can grow at a wide range of temperatures. It is aerobic and facultatively anaerobic. Colonies grown on agar media are raised, smooth, glistening, gray and circular in outline. Heavily encapsulated strains appear mucoid, rough colonial forms also occur. Some strains are beta hemolytic on blood agar (Hagan and Timoney, 1988).

E. coli doesn't liquefy gelatin and does not use citrate, it ferments glucose and other carbohydrates by means of the conversion of pyruvate into lactic acid. Most strains ferment lactose (Hagan and Timoney, 1988).

E. coli forms indole usually in abundance; vigorously reduces nitrates; and reacts negatively to the Voges-Proskauer test. The Voges-Proskauer test is valuable in distinguishing *E. coli* from *Enterobacter* aerogenes which reacts positively (Hagan and Timoney, 1988).

1-5-1 Media for Isolation of E. coli:-

- Differential and Selective Media:-

A - MacConkey's Agar Medium:-

It is used to detect coliform and enteric bacteria from faecal samples based on their ability to ferment lactose. Lactose fermenting bacterial species like *E. coli* gives pink to red colonies while other non lactose fermenting organisms give colorless to transparent colonies after an overnight incubation at 37˚C on this medium (Barrow and Feltham, 1993).

B - Chromogenic E. coli/coliform Medium:-

This medium is used for the differentiation between *E. coli* and other coliforms in cultures produced from food samples. *E .coli* produced purple colonies while coliforms have pink colonies (Ronald, 2006)

- Enriched Media:-

Blood agar is used for primary isolation of *E. coli* from systemic infections.

- Basic Media:-

Nutrient agar is used for subculturing of *E. coli* from differential selective or storage media prior to biochemical and serological identification (Cheesbrough, 2002).

1-5-2 Biochemical Tests:-

Biochemical tests for the differentiation of *E. coli* from other closely related bacterial groups must be based on the reactions which occur in a variety of media. All strains of *E*. *coli* ferment glucose and lactose with the production of acid and gas (Buxton and Frazer, 1977).

In order to distinguish *E. coli* from related species a battery of tests called the IMViC reactions was developed in order to differentiate fecal coliforms from non fecal coliforms. IMViC is an acronym in which the capital letters stand for:-

1- Indole.

2- Methyl red.

3- Voges-Proskauer.

4- Citrate.

The IMViC set of tests examines the ability of an organism to produce indole, produce sufficient acid to change the color of methyl red indicator, produce acetoin, an intermediate in the butanediol fermentation pathway (a positive result of the Voges-Proskauer test); and the ability to grow on citrate as the sole source of carbon. *E. coli* is positive in the first two tests and negative in the second two (Todar, 2005).

1-6 Maintenance and Preservation:-

E.coli can survive well in holding media as modified Carey-Blair medium for several weeks to one month without losing its plasmids. Storage of strains in liquid broth media supplemented with 15% glycerol as cryopreservative at -70˚C, gives good stability of the enterotoxin properties as well as of the surface adhesin. Storage of strains on Dorset egg medium at 4˚C is a good alternative for liquid broth media. Lypholization of *E. coli* strains also give a good stability of plasmid for years (Sack, 1981).

1-7- Pathogenic *Escherichia coli*:-

Six clinically distinct categories (pathovars) of disease-causing *E. coli* have been described. The different virulence and infective characteristics are derived from a combination of the factors determining surface adsorption and adherence of epithelium and the toxins that may or may not be produced (Harrigan, 1998). The six types are:-

1- **Enteropathogenic *E. coli* (EPEC):-**

EPEC doesn't produce high level of shiga-like toxins, but attach to, and efface micro villi. Adherence of EPEC strains to the intestinal mucosal is a very complicated process and produces dramatic effects in the ultrastructure of the cells resulting in rearrangement of action in the vicinity of adherent bacteria. The phenomenon is sometimes called "attaching and effacing" of cells. EPEC strains are said to be "moderately invasive" meaning they are not as invasive as *Shigella*, and unlike enterotoxigenic E. coli (ETEC) or enteroaggergative (EaggEC), they cause an inflammatory response. The diarrhea and other symptoms of EPEC infection probably are caused by

bacterial invasion of host cells and interference with normal cellular signal transduction, rather than proguction of toxin (Todar, 2005).

Sero vars include O55, O86, O111, O119, O125, O126, O127, O128ab, O142 and O158 (Harrigan, 1998). Some types of EPEC are referred to as Enteroadherent *E. coli* (EAEC), based on specific patterns of adherence they are an important cause of traveler's diarrhea in Mexico and in North Africa (Harrigan, 1998).

2- Enterotoxigenic *E. coli* (ETEC):-

ETEC are an important cause of diarrhea in infants and travelers in under developed countries or regions of poor sanitation. The diseases vary from minor discomfort to a severe cholera-like syndrome. ETEC are acquired by ingestion of contaminated food or water, and adults in endemic areas evidently develop immunity (Todar, 2005). ETEC Possess particular colonization factor antigens and also produces heat-labile (LT) toxin and heat-stable (ST) toxin. serovar include O6, O8, O15, O20, O25, O27, O63, O73, O78, O80, O114, O115, O128ac, O139, O148, O149, O153, O159, O166 and O169 (Harrigan, 1998).

Enterotoxins produced by ETEC include LT toxin and/or ST toxin, the genes for which may occur on the same or separate plasmids. The LT enterotoxins are similar to cholera toxin in both structure and mode of action. LT toxin binds to the same intestinal receptors that are recognized by the cholera toxin, and its enzymatic activity is identical to that of the cholera toxin (Todar, 2005).

The ST enterotoxin is actually a family of toxins which are peptides of molecular weigh about 2,000 Daltons. Their small size explains why they are not inactivated by heat. ST causes an increase in cyclic GMP in host cell cytoplasm, this leads to secretion of fluid and electrolytes resulting in diarrhea. Symptoms of ETEC infections include diarrhea without fever. The bacteria colonize the gastrointestinal tract by means of a fimbrial adhesion e.g. CFA1 and CFA2, and are non invasive (Todar, 2005).

3-Enteroinvasive *E. coli* (EIEC) and *Shigella*:-

They genetically nearly identical and have a number of antigens in common. In fact, in Bergey's Manual it is suggested that *E. coli* and the four species of *Shigella* spp form a single species on the basis of DNA relatedness, and *Shigella* are merely metabolically inactive biogroups of *E. coli*. EIEC serovars include O28ac, O29, O112, O124 (equivalent to *S. dysenteriae* 3), O136, O143 (equivalent to *S. boydii* 8), O144, O152 (equivalent to *S. dysenteriae* 12), O164 and O167 (Harrigan, 1998).

The clinical syndrome is identical to *Shigella* dysentery and includes a dysentery-like diarrhea with fever.

4- Verocytotoxin-producing *E. coli* (VTEC) or Enterohaemorrhagic *E. coli* (EHEC):-

VTEC or EHEC strains of *E. coli* that produces a cytotoxin against vero cells (a tissue culture cell line from African green monkey kidney). They were first described in 1977 (Harrigan. 1998).

EHEC is represented by a single strain (serotype O157:H7), which cause a diarrheal syndrome distinct from EIEC (and *Shigella*) in that there is copious bloody discharge and no fever. A frequent life-threating situation is its toxic effect on the kidneys (hemolytic uremia). EHEC produce a toxin that is virtually identical to the Shigella toxin. The toxin plays a role in the intense inflammatory response produced by EHEC strains and may explain the ability of EHEC strains to cause hemolytic uremia. This toxin is phage encoded and its production is enhanced by iron deficiency (Todar, 2005).

5-Entero aggregative E. coli (EaggEC):-

The distinguishing feature of EaggEC strains is their ability to attach to tissue culture cells in an aggregative manner. These strains are associated with persistent diarrhea in young children. They resemble ETEC strains in that the bacteria adhere to the intestinal mucosa and cause non-bloody diarrhea without invading or causing inflammation. This suggests that the organisms produce a toxin of some different sort (Todar, 2005).

Recently, a distinctive heat-labile plasmid-encoded toxin has been isolated from these strains, called the EAST (EnteroAggergative ST) toxin. They also produce a hemolysin related to the hemolysin produced by *E. coli* strains involved in urinary tract infection (Todar, 2005).

The self-adherent tending to autoagglutination, giving the appearance under the microscope of a stack of brick. These organisms, their serological characterization and the clinical diseases they cause have yet to be fully described (Harrigan, 1998).

6- Diffusively Adherent E. coli (DAEC):-

DAEC are even less well characterized than EaggEC (Harrigan 1998).

Pathogenic strains of *E. coli* excrete at least five medically important products including:-

1- Enterotoxins:-

Enterotoxins are usually plasmid-encoded proteins and occur in two forms one labile toxin (LT), is a large heat-labile immunogenic protein of 91.000MW that is antigenically related to cholera toxin. The other, stable toxin (ST) is a family of non immunogenic proteins, 1500 to 2000MW. These protein exotoxins affect the regulation of cyclic nucleotide activity within the cell (Hirsh and Yuanchang, 1999).

2- Sidrophores:-

These products allow microorganisms to acquire iron from the host; microorganisms must acquire iron from the environment. To multiply within the host, microorganisms must acquire iron from the host iron-binding proteins because there is little free iron within the host. Sidrophores that remove iron from host iron-binding protein are necessary if a microbe is to have invasive capabilities (Hirsh and Yuanchang, 1999).

3- Shiga-like toxins:-

These are protein toxins similar in activity to Shiga toxins produced by *Shigella.* Both Shiga toxins and Shiga-like toxins inhibit protein synthesis following interaction with the 60s ribosomal subunit (Hirsh and Yuanchang, 1999).

4- Cytotoxic Necrotizing Factors (CNF):-

E. coli may produce (CNF), which interacts with an epithelial cell small GTP-binding protein Rho, resulting in membrane "ruffles". There are two types of CNF; CNF1 and CNF2, which are immunologically related and similar in size (Hirsh and Yuanchang, 1999).

5- Hemolysins:-

E. coli produces at least three hemolysins (α- hemolysis, enterohemolysin "Ehx" for EHEC and cytolysin A "Cly"). The alpha hemolysin is a protein secreted by many virulent strains of *E. coli*. The hemolysins damages cell membranes (Hirsh and Yuanchang, 1999)

1-8- *E. coli* Infections in Animals:-

As a general rule, the acute infections of neonatal animals characterized by bacteremia or septicemia are caused by invasive strains of *E. coli*, while the diarrheal infections are due to enterotoxin-producing strains (enteropathogenic or enterotoxigenic) (Carter, 1986).

Colibacillosis is a general term that denotes *E. coli* infections characterized by one or more of the following: diarrhea, enteritis, septicemia, or bacteremia. Rota and corona viruses may be involved as well as *E. coli* (Carter, 1986).

a- Colibacillosis in Farm Animals:

It's caused by pathogenic serotypes of *E. coli*. The prevalence of the different pathogenic serotype of *E. coli* in farm animals has remained relatively unchanged for many years. Certain serotypes cause diarrhea and others cause septicemia, the most common enteropathogens which cause diarrhea in new born farm animals are the enterotoxigenic *E. coli* (ETEC). Enterohemorrhagic *E. coli* are an uncommon cause of disease of new born farm animals and attach to colon and distal small intestine (Radostits, 2000).

Attaching and effacing strains *E. coli* cause hemorrhagic colitis, and also known as verocytotoxic *E. coli* because they produce verocytoxin. Necrotoxigenic *E. coli* (NTEC) produce cytotoxic necrotizing factor1 (CNF1) or 2 (CNF2), NTEC2 isolates are restricted to calve and lambs with diarrhea and septicemia (Radostits, 2000).

Colibacillosis occurs most commonly in new born farm animals and is a significant cause of economic loss in raising live stock. It is a complex disease

in which several different risk factors interact with certain pathogens resulting in disease (Radostits, 2000).

There are at least two different types of the disease; enteric colibacillosis is characterized by varying degrees of diarrhea, dehydration, acidosis and death in a few days if not treated; coliform septicemia is characterized by severe illness and rapid death in several hours (Radostitis, 2000).

Morbidity rates in dairy calves raised under intensified and poorly managed conditions reaches 75% but is usually about 30%. Case fatality rates vary from 10 – 50% depending on the level of clinical management. In beef calves the morbidity rate vary from 10 – 50% and the case fatality rate from 5 – 25% or even higher in some years (Radostits, 2000).

b- Mastitis:

E. coli mastitis can develop in any host species but is most common in dairy cattle, in which the disease has been noted particularly in high-producing animals, in first two weeks after calving and in animals with low somatic cell counts. Infection of mammary gland by *E. coli* results in mild to severe inflammation and a clinical course of mastitis that may be peracute, acute, or chronic. The *E. coli* strains that are implicated in mastitis are the *E. coli* strains that found in the environment. And these organisms do not appear to possess any special virulence factors (Hill, 1994).

Environmental *E. coli* that contaminate the teat orifice make their way through the streak canal into the gland lumen, where the host response determines the clinical outcome. Cows are most likely to become infected when they lie in faeces-contaminated bedding after they have been milked and the teat orifices remain open, experimentally; as few as 60 bacteria are sufficient to cause mastitis (Carlton, 2004).

c- Colibacillosis in Poultry:

Refers to any localized or systemic infection caused entirely or partly by avian pathogenic *E. coli* (APEC) including: eolisepticemia, coligranuloma (Hjarre's disease), air sac disease (chronic respiratory disease, (CRD, coliform cellulitis, swollen-head syndrome, coliform peritonitis, coliform salpingitis, coliform osteomyelitis/synovitis. Colibacillosis in mammals most often a primary enteric disease; whereas colibacillosis in poultry is typically a

localized and systemic disease occurring secondarily when host defenses have been impaired or over whelmed by virulent *E. coli* strains (Saif, 2003).

1-9- E. coli Infection in Human:-

E. coli is responsible primarily for three types of infections in humans: urinary tract infections, neonatal meningitis, and intestinal diseases. These conditions depend on a specific array of pathogenic (virulence) determinants possessed by the organism (Todar, 2002).

- Urinary Tract Infections:

Uro-pathogenic *E. coli* causes 90% of the urinary tract infections in anatomically-normal, unobstructed urinary tract. The bacteria colonize from the feces or perineal region and ascend the urinary tract to the bladder. Bladder infections are 14 times more common in females than male by virtue of the shortened urethra (Todar, 2002).

- Neonatal Meningitis:

Neonatal meningitis affects 1/2000 – 4000 infants. Eighty percent of *E. coli* strains involved synthesize K-1 capsular antigens (K-1 antigen is only present 20- 40% of the time in intestinal isolates). *E. coli* strains invade the blood stream of infants from the nasopharynx or gastrointestinal tract and carried to the meninges. The K-1 antigen is considered the major determinant of virulence among strains of *E .coli* that cause neonatal meningitis. K-1 is a homopolymer of sialic acid, its inhabits phagocytosis, complement, and responses from the host's immunological mechanisms. K-1 may not be the only determinant of virulence; however, as sidrophore production and endotoxin are likely to be involved. Neonatal meningitis requires antibiotic therapy that usually includes ampicillin and a third-generation cephalosporin (Todar, 2002).

- Intestinal Diseases:

As a pathogen, *E. coli* of course, is best known for its ability to cause intestinal disease. Five classes (virotypes) of *E. coli* that cause diarrheal disease are now recognized: enterotoxigenic *E. coli* (ETEC) affects small intestine and cause Traveler's diarrhea characterized by watery stool, cramps, nausea, and mild fever. enteroinvasive *E. coli* (EIEC) affects large intestine and causing *Shigella*-like diarrhea characterized by fever, cramps, watery

diarrhea followed by scant, bloody stool. enterohemorrhagic *E. coli* (EHEC) affects large intestine cause hemorrhagic colitis, severe abdominal pain, watery diarrhea followed by grossly bloody stool. Enteropathogenic *E. coli* (EPEC) affects small intestine and causes infantile diarrhea (Salmonella-like) with fever, nausea,and vomiting, and lastly enteroaggregative *E. coli* (EAggEC). Each class falls within a serological sub-group and manifests distinct features in pathogenesis (Todar, 2002 and Douglas, 2007).

1-10- Immunity to *E. coli* Infections in Animals:

Immunity to enteric *E. coli* infection is based on the presence in intestinal tract of antibodies to surface antigens. The most widely investigated of theses antibodies are antifimbriae antibodies, which have been shown to be protective when they are delivered to the intestine of nursing pigs or calves through colostrums and milk of vaccinated dams (Carlton 2004). The antibodies may also be delivered in the feed of weaned animals (Imberecht *et al*., 1997). Antibodies to the polysaccharide capsule of ETEC may also be protective. Antifimbriae and anti-K antibodies function by preventing attachment of the ETEC to enterocytes. Where as much success has been achieved in protecting nursing animals through vaccination of the dam. The loss of milk antibodies renders weaned animals susceptible to infection.

Control of *E. coli* mastitis is based primarily on reducing contamination of udder, but a bacterin consisting of killed *E. coli* strain J5, a rough mutant of an O111 *E. coli*, has also been used to provide protection against severe mastitis (Carlton 2004). Vaccine that blocks the bacterial enterochelin system for obtaining iron in a low iron environment induced antibodies that inhibited the growth of *E. coli* (Carlton, 2004). The vaccine consisted of FepA, a protein on the bacterial surface that acts as a receptor for iron-enterobactin complexes used by *E. coli* in the mammary gland to bring iron into the cell. It has been suggested that this vaccine may be effective in protecting cows against *E. coli* mastitis, but it is possible that *E. coli* may be able to use an alternative system. Vaccination of cattle with FecA, the receptor for the ferric citrate iron acquisition system, resulted in a reduction in clinical severity of experimentally induced mastitis and in IgG that reduced uptake of iron by *E. coli* in a reduced iron environment (Carlton, 2004).

1-11- Escherichia coli O157:H7:

1-11-1- Classification, Definition, and Historical Background for *E. coli* O157:H7:

E. coli O157:H7 is a specific serotype of *E. coli* that cause watery diarrhea, hemorrhagic colitis and hemolytic uremic syndrome in humans. *E. coli* O157:H7 is the most important serotype of the shiga-toxin-producing *E. coli* (STEC), named for it somatic (O) and flagellar (H) antigens (Sherwood *et al*., 2004).

E coli serotype O157:H7, which belong to enterohaemorrhagic group (EHEC), is a rare variety of *E. coli* that produces large quantities of one or more related potent toxins that cause severe damage to the epithelial lining of the intestine (Fegan and Desmarchelier, 1999).

Enterohaemorrhagic *E. coli* strain are a recently emerged group of food-borne pathogens that are a significant public health threat, due mainly to the severity of clinical outcomes (Karch *et al.,* 2005; WHO, 2005). *E. coli* O157:H7 is the most clinically relevant serotype of enterohaeorrhagic *E. coli* strains in most industrialized countries, including the United State of America (Armstrong *et al*., 1996; Karch *et al*., 2005). It's significant as a public health problem was recognized in 1982, following an outbreak in the united State of America. EHEC produce toxins, known as verotoxins or a shiga-like toxin because of its similarity to toxins produce by *Shigella dysenteriae* (WHO, 2005).

The recognition of EHEC as a distinct class of pathogenic *E. coli* resulted from two key epidemiologic observation. The first reported in 1983, by Riley *et al*., who investigated two outbreaks of a distinctive gastrointestinal illness characterized by severe cramp abdominal pain, watery diarrhea, and little or no fever. This illness, designated as hemorrhagic colitis (HC), was associated with the ingestion of undercooked hamburgers at fast-food restaurant chain. Stool cultures from these patients yielded a previously rarely isolated *E. coli* serotype O157:H7. The second key observation was by Karmali *et al*. (1983), who reported the association of sporadic cases of

hemolytic uremic syndrome (HUS) with fecal cytotoxin and cytotoxin-producing *E. coli* in stools. HUS (defined by the triad of acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia) was already known to be preceded typically by a bloody diarrheal illness indistinguishable from HC. The cytotoxin assay used by Karmali *et al*. (1983) was originally reported by Konowalchuk and colleagues (1977). These investigators reported that culture filters from some strains of *E. coli* produced a striking, irreversible cytopathic effect on culture vero cells that was quite distinct from the noncytopathic effect of ETECLT or CHO or y-1 cells. At this same time, O'Brein *et al*. (1982, 1983) reported that extract of certain *E. coli* strains were cytotoxic for Hella cels and that this cytotoxic activity could be neutralized by antitoxin prepared against crude *Shigella dysenteriae* (shiga) toxin (stx). They subsequently reported that many *E. coli* strains isolated from diarrheal illness produced shiga-like toxin (SLT0, including one of the strain reported by Konowalchuk et al, (1977) to produce the verocytotoxin (O'Brein et al., 1982). Subsequently have been showed that shiga-like toxin and the verocytotoxin were the same toxin and O157:H7strains described by Riley et al, produced this toxin (O'Brien *et al*., 1983).

Therefore, it appears that the presence of O157:H7 strains has genuinely increased in recent years and not simply missed prior to 1982. However, HUS was well-known clinically entity prior to 1982. Since its initial description in 1955, numerous outbreaks of HUS gave credence to the hypothesis that HUS was due to a bacterial or viral agent (Karmali 1989). Although stx-producing *S. dysenteriae1* strains were clearly associated with HUS, stool cultures obtained during many HUS outbreaks yielded *E. coli* but no recognized pathogens. In a remarkably prescient 1968 article describing HUS in South Africa (Kibel, and Barnard 1968) suggested that a mutant strain of *E. coli* mutated by a bacteriophage may be responsible for this syndrome. In 1980s it was recognized that stx encoded on a bacteriophage in *E. coli* (O'Brein *et al*., 1984; Scotland et al., 1983 and Smith et al., 1993) and that over 100 different *E. coli* serotypes can express stx (Karmali 1989).

1-11-2- Biochemical Characteristic and Cultural Requirements of *E. coli* O157:H7:

There are some biochemical characteristics of *E. coli* O157:H7 that have been exploited in the isolation and identification of this serotype. An important characteristic is that O157:H7 strains do not ferment D- sorbitol rapidly, in contrast to about 75 to 94% of other *E. coli* strains (March and Rantnam, 1986; Farmer *et al*., 1995; and Farmer *et al*., 1985) are rapidly ferment sorbitol. *E. coli* O157:H7 strains also do not ferment rhamnose on agar plates, whereas 60% of non-sorbitol-fermenting *E. coli* belonging to other serogroups ferments rhamnose (Smith *et al*., 1993).

Another characteristic of *E. coli* O157:H7 that distinguishes it from most other serotypes of *E. coli* is the inability to produce β-glucuronidase, which hydrolyze 4-methyl-umbeliferyl-D-glucuroide (MUG) and related substrates (Thompson *et al*., 1990).

E. coli O157:H7 also doesn't grow well at 44 to 45.5˚C, which is a temperature commonly used to grow *E. coli* from food and water samples (Proulx *et al*., 1992). EHEC strains can grow in temperatures ranging from 7˚C to 50˚C, with an optimum temperature of 37˚C. Some EHEC can grow in acidic foods, down to a pH of 4.4 (WHO, 2005).

1-11-3- Epidemiology:

Occurrence:

Human infections with *E. coli* O157:H7 are distributed worldwide. Cases have been reported from several countries in North America, Europe, Africa, eastern Asia and Australia. North America, Argentina and the United Kingdom are regions with a higher frequency of known infection (George, 1994).

In United States where the first cases were confirmed in 1982, population surveys made in 1985 -1986 led to an isolation rate of 8.0/100,000 and an infection rate of 2.1/100,000 annually (George, 1994).

E. coli O157:H7 has been the cause of hemorrhagic colitis in 15% - 36% of cases in the USA and 39% of cases in UK, sporadic cases of hemorrhagic colitis and hemolytic uremic syndrome were reported in Germany and other countries , mostly northern countries (George, 1994).

Reservoir:

Cattle seem to be the main source of *E. coli* O157:H7; the first outbreaks were traced to ground beef. Later several cases of infection transmitted by raw milk were reported. Isolation were made mainly from calves and heifers but also from dairy cows, especially young animals and beef cattle (George, 1994).Stx-producing *E. coli* can be found in the fecal flora of a wide variety of animals including cattle, sheep, goats, pigs, cats, dogs, chickens and gulls (Beutin et al., 1993; Griffin and Tauxe, 1991; Johnson *et al*., 1993; and Wallace *et al*., 1997), other ruminants reservoirs including camels (WHO, 2005).

Calves infected with *E. coli* O157:H7 may become clinically ill, but mostly the animals harbor the organisms in their intestine as in apparent carriers. *E. coli* O157:H7 may colonize chicken ceca and turkey meat, but hither to food borne infections have been traced only to food of bovine origin (George, 1994).

The reservoir of EHEC in farm animals is further documented by seroepidemiology surveys, which show that the incidence of elevated O157LPS antibody levels in serum is three fold higher in Canadian dairy farm families than in urban families (Reymond *et al*., 1996).

Prevalence:

Susceptibility of people to development of hemolytic anemia and (HUS) after infection with *E. coli* O157:H7 differs. Enhanced risks exist for children less than 5 years of age and immunocompromised or old people. Some studies have found that female sex is a risk factor (George, 1994).

The incidence of *E. coli* O157:H7 infections vary by age group, with the highest incidence reported case occurring in children aged less than 15 years (0.7 cases per 100000 in the US). Sixty-three to 85% of cases are a result of exposure to the pathogen through food. The percentage of *E. coli* O157:H7 infection which progress to HUS varies between sporadic cases (3% -7%) and those associated with outbreaks (20% or more). In epidemiological term there is generally a background of sporadic cases, with occasional outbreaks. Some of these outbreaks have involved a high number of cases, such as in Japan 1996, where an outbreak linked to contaminated radish sprouts in school lunches causes 9451 cases (WHO, 2005).

Susceptibility of animals seem to be low, clinically significant infection are known in calves in Argentina and Spain, but the surveys of calves with diarrhea in USA and UK did not lead to isolations of *E. coli* O157:H7.

Transmission:

The principle method of transmission in an outbreak by *E. coli* O157:H7 has been consumption of under cooked ground beef. Unpasteurized milk has caused hemorrhagic colitis in infants. In USA some outbreaks of hemorrhagic colitis were transmitted by water contaminated with *E. coli* O157:H7, as with other *E. coli* strains, O157:H7 is found in water contaminated by feces, e.g. with the effluents from cattle farms and abattoirs or from community sewage (George, 1994).

Many infections resulted not from direct ingestion of undercooked hamburgers but from cross-contamination of other food items by food preparers who did not wash their hands after handling row ground beef. Other foods of bovine origin, including roast beef and raw milk, and other types of meat including meat from porcine, avian and sheep sources, have also been directly linked to outbreaks (Griffin and Tauxe 1991; Griffin 1995).

The spectrum of vehicles implicated in disease due to EHEC is expanding for beyond the initial hamburger-associated outbreak. Recent outbreaks have been linked to consumption of mayonnaise (Griffin 1995), unpasteurized apple juice (Besser *et al*., 1993; and MacCarthy, 1996), and fermented hard salami (Center for Disease control, 1995). The last two vehicles illustrate a notable ability of *E. coli* O157:H7 to grow in food of low pH. The organism can adapt to acidic condition to allow it to survive at pH 3.4 for several days (Benjamin and Datta, 1995; Li *et al*., 1993; and Zhang and Donnehberg 1996).

Raw vegetables such as lettuce have been the incriminated vehicle in several outbreaks (Morgan *et al*., 1988). In most of these cases the fruits or vegetables were believed to be contaminated with cattle feces.

Person-to-person contact is an important mode of transmission through the oral-fecal-route. An asymptomatic carrier state has been reported, where individuals show no clinical signs of disease but are capable of infecting others. The duration of excretion of *E. coli* O157:H7 is about one week or less in adult but can be longer in children (WHO, 2005).

In United States and Canada the most cases have occurred in the warmer summer months. In the United Kingdom in 1991, infections with O157:H7 showed a peak incidence in the third quarter of the year (George, 1994).

1-11-4- Pathogenesis and Virulence Factors of *E. coli* O157:H7:

Most of the work on pathogenic factors of *E. coli* O157:H7 has focused on the Stx, which are encoded on a bacteriophage inserted into the chromosome. Additional potential virulence factors are encoded in the chromosome and on a Ca60-MDa plasmid found in all EHEC strains of serotype O157:H7. Orally ingested *E. coli* producing Stx must initially survive the harsh environment of the stomach and then compete with other gut microorganisms to establish intestinal colonization. STEC organisms remain in the gut, and so Stx produced in the lumen must be first adsorbed by the intestinal epithelium and then translocated to the blood stream. This permits delivery to the specific toxin receptors on target cell surfaces inducing both local and systemic effects (James and Adrienne, 1998). Having survived the harsh conditions of stomach, STEC must establish colonization of the gut by adhering to intestinal epithelial cells. It's generally assumed that the colon and perhaps also the distal small intestine are the principle sites of STEC colonization in humans (James and Adrienne, 1998). The classic intestinal histopathology characteristic of *E. coli* O157:H7 infections include hemorrhage and edema in lamina propria, colonic biopsy specimens from many patients also show focal necrosis and infiltration of neutrophils (Griffin *et al*., 1990).

The major virulence factor, and a defining characteristic of EHEC, is Stx this potent cytotoxin is the factor that leads to death and many other symptoms in patient infected with EHEC (O'Brien and Holmes 1987, 1996; O'Brien *et al*., 1992; Sears and Kaper, 1996; Taylor *et al*., 1991).

The Stx family contains two major immunologically non-cross-reactive groups called Stx1 and Stx2. a single EHEC strain may express Stx1 only, Stx2 only, or both toxins or even multiple forms of Stx2. Stx1 from EHEC is identical to shiga toxin from *S. dysenteriae1* (Jarvis *et al.,* 1995). There is a variety of data showing the involvement of Stx in diarrhea and enteric colitis, beginning with early demonstrations that purified Stx can cause fluid

accumulation and histologyical damage when injected into ligated intestinal loops (O'Brien and Holmes 1987). One possible mechanism for fluid secretion in response to Stx involves the selective killing of absorptive villus tip of intestinal epithelial cells by Stx (Kandel *et al.,* 1989; Keenan *et al.,* 1986).

Stx produced in the intestine is assumed to translocate the blood stream, although toxin has been detected in the blood of HUS patients. In polarized intestinal epithelial cells in vitro, Stx moves across the epithelial cell monolayer without obvious cellular disruption, probably through a transcellular, rather than paracellular, pathway (Acheson *et al*., 1992). Damage of the intestinal epithelium by Stx, bacterial lipopolysaccharide (LPS), or other inflammatory mediators could also aid translocation of the toxin to the blood stream. This possibility is supported by the fact that patient with bloody diarrhea due to *E. coli* O157:H7 are more likely to develop HUS than those with non bloody diarrhea (Griffin, 1995).

Epidemiological data suggested that Stx2 is more important than Stx1 in the development of HUS (Griffin, 1995). Although the simplest mechanism for HUS involves direct cytotoxin action of Stx on renal endothelial cells, there are also several studies that support a role for cytokines in this process (Nataro and Kaper, 1998).

Intestinal adherence factor, the only potential *E. coli* O157:H7 adherence factor that has been demonstrated to play a role in intestinal colonization in vivo in an animal model is the 94-to 97-kDaOMP intimin encoded by the eae gene (Nataro and Kaper, 1998).

All strains of O157:H7 contain a highly conserved plasmid, designated PO157 (Schmidt *et al.,* 1994), which varies in size from 93.6 to 104 kb (Schmidt *et al*., 1996). This plasmid is also present in O26:H11 strains and is present in most but not all Stx-producing *E. coli* strains isolated from humans (Beutin *et al.,* 1994; Levine *et al.,* 1987). This plasmid encoded a catalase-peroxidase, whose function is unknown (Brunder *et al.,* 1996). A possible role of this plasmid in the suppression of production of an expolysaccharid has also been suggested (Fratamico *et al.,* 1993). A 3.4 kb fragment of this plasmid was developed by Levine *et al*., (1987) as a diagnostic probe for EHEC. The role of this plasmid in the pathogenesis of disease due to EHEC is unknown. Epidemiological evidence suggested a stronger correlation of the

presence of this plasmid with the development of HUS rather than diarrhea, the enterohemolytic phenotype encoded on this plasmid was observed in 16(88%) of 18O111: H-strains isolated from patients with HUS but in only 4(22.2%) of 18 O111: H-strains isolated from patients with diarrhea without HUS (Schmidt *et al.,* 1996).

E. coli O157:H7 contains a specialized iron transport system which allows this organism to use heme or hemoglobin as an iron source (Law and Kelly, 1995; Mills and Pyne*,* 1995; Torres *et al*., 1997). The growth of *E. coli* O157;H7 is stimulated by presence of heme and hemoglobin, and the lysis of erythrocytes by one or more of the hemolysins reported for this pathogen could release these sources of iron, thereby aiding infection (Law and Kelly, 1995; Mills and Pyne*,* 1995),.

Other potential virulence factors O157LPS as well as LPS from other bacteria enhance the cytotoxicity of Stx on human vascular endothelial cells in vitro, but its effects in vivo are not clear. (Oelschlaeger *et al*., 1994) reported that *E. coli* O157:H7 can invade cultured intestinal cell lines, but a later report (Mckee and O′Brien, 1995) disputed these findings, showing that O157:H7 strains were no more invasive than *E. coli* strains from the normal flora, there is no in vivo evidence that invasion occurs in human or animals.

1-11-5- Diseases Caused by *E. coli* O157:H7:

Symptoms of the disease caused by *E. coli* O157:H7 including; abdominal cramps and diarrhea that may in some cases progress to bloody diarrhea (hemorrhagic colitis). Fever and vomiting may also occur, the incubation period can range from three to eight days, most patients recover within 10 days, but in a small proportion of patients (particularly young children and elderly), the infection may lead to a life-threatening disease, such as hemolytic uremic syndrome (HUS) (WHO, 2005).

HUS is characterized by acute renal failure, hemolytic anemia and thrombocytopenia. It is estimated that up to 10% of patients with hemorrhagic colitis develop HUS, with a case-fatality rate ranging from 3% to 5%over all; HUS is the most common cause of acute renal failure in young children. It can cause neurological complications (such as seizure, stroke and coma) in 25% of HUS patients and chronic renal squeal, usually mild in a round 50% of survivors (WHO, 2005).

1-11-6- E. coli O157:H7 Infections Control and Preventive Measures:

The prevention of infection requires control measures at all stages of food chain, from agricultural production on the farm to processing, manufacturing and preparation of food in both commercial establishment and the domestic environment. Good hygienic slaughtering practices reduce contamination of carcasses by feces, but do not guarantee the absence of *E. coli* O157:H7 from products. Education in hygienic handling of foods for abattoir workers and those involved in the production of raw meat is essential to keep microbiological contamination to a minimum. Similarly prevention of contamination of raw milk on the farm is virtually impossible, but the education of farm workers in principles of good hygienic practice should be carried out in order to keep contamination to a minimum. The effective method of eliminating *E. coli* O157:H7 from foods is to introduce a bactericidal treatment, such as heating (cooking or pasteurization) or irradiation (WHO. 2005).

Prevention of infections of cattle and other animals seem to be difficult because the method of infection of these animals has not been fully clarified. The greatest success in prevention of infections of animals with *E. coli* O157:H7 may be achieved by controlling their drinking water. *E. coli* O157:H7 is known to be transmitted by water contaminated by feces. Effluents from farms, abattoirs, and community waste water may contain *E. coli* O157:H7 and should not have direct contact with surface water (George, 1994).

1-12- Antibiotic Sensitivity:

Antibiotic usage is consideried the most important factor promoting the emergence, selection and dissemination of antibiotic-resistant micro-organisms in both veterinary and human medicine. Antibiotic usage selects for resistance not only in pathogenic bacteria, but also in the endogenus flora of exposed individuals or populations (Bogaard *et al.,* 2000).

Currently, there is increase public and scientific interest in the use of therapeutic and sub-therapeutic antimicrobials in animal. This due primarily to the possible emergence and dissemination of multiple-drug-resistant zoonotic bacterial pathogens via transmission as food pathogens (David *et al.,* 2000).

Multiple-drug-resistance in enteric organisms like *E. coli* is known to be associated with integron, which contain gene and a cassettle integration

site, into which antibiotic resistance gene cassettle has integrated (Lydia *et al.,* 1999).

The susceptibility to different *E. coli* isolates to different antibiotics at the specified concentration was studied (Mohamed, 2006) who reported that , all *E. coli* strains isolated were resistant to one or more of beta-lactam antibiotics and multi-drug resistance was shown by most of *E.* coli strains.

CHAPTER Two

MATERIAL AND METHODS

2-1 Samples for Bacteriological Examination:-

2-1-1 Fecal Samples:-

A total of 45 fecal samples were collected from healthy animals, 15 from cattle, 15 from sheep and 15 from goats. They were obtained from Alkalakla, Kuku and Shambat localaties in Khartoum State either from animal's farms or house yards.

2-1-2 Rectal swabs:-

A total of 60 rectal swabs, 45 were taken from the same previous animals and 15 from poultry, were collected in the same previous areas.

2-1-3 Collection of samples:-

Faeces (>1g) were collected from cattle, sheep and goats. Each sample was collected into sterile polystyrene universal container.

Rectal swabs were collected from cattle, sheep, goats and poultry. Sterile swabs were inserted into the rectum or cloaca.

All samples were labeled and transported to the laboratory at ambient temperature, and always tested within 2h of collection.

2-2 Culture Media:-

2-2-1 Liquid Media:-

A- Peptone Water (Oxoid):-

Fifteen grams of dehydrated peptone water medium were added to 1 liter of distilled water and mixed well to dissolve. The pH was adjusted to 7.2; then distributed into sterile test tubes in 10 ml and sterilized by autoclaving at 121˚Cfor 15 min.

B- Peptone Water Sugars (Barrow & Feltham, 1993):-

The pH of the peptone water was adjusted to 7.1-7.3 before the Andrade's indicator was added and then 10 g of appropriate sugar were added to the peptone-indicator mixture, mixed thoroughly and then distributed into sterile test tubes, about 5 ml each, with or without inverted Durham's tubes. Then, the tubes were sterilized by autoclaving at 110˚C for 10 min.

C- Glucose-phosphate Medium (Barrow & Feltham, 1993):-

Ingredients (see appendix) were steamed until the solids were dissolved in 1 liter of distilled water; the pH was adjusted to 7.5. 5g of glucose were added, mixed and distributed in 1.5 ml volumes into sterile Bijou bottles. Then sterilized at 115˚C for 10 min. This medium is used for the MR and VP test.

D- Malonate Broth (Difco code NO 0395-01):-

The solid ingredients (see appendix) were dissolved in 1 liter of distilled water by heating (boiling). The pH was adjusted to 7.0 before filtration of the media. Then, the indicator solution was added and the media was distributed into sterile test tubes in 5 ml amounts and sterilized by autoclaving at 115˚C for 20 min.

E- Nutrient Broth (Biomark, code NO B274):-

Thirteen grams of dehydrated medium were suspended in 1000 ml of distilled water and boiled to dissolve, the pH was adjusted to 7.2-7.6 and the preparation was then distributed into the final containers and sterilized by autoclaving at 121˚C for 15 min.

2-2-2 Semi- solid Media:-

A- Hugh and Leifson's (O-F) Medium (Barrow & Feltham, 1993):

The solid materials (see appendix) were dissolved by heating in 1 liter of distilled water. The pH adjusted to 7.1, the indicator was added. The medium was sterilized at 115c◦ for 20min. Glucose was added aseptically to give final concentration of 1%. Then the medium was mixed and distributed aseptically in 10ml volumes into sterile tubes.

B- Motility Medium (Barrow & Feltham, 1974):-

14.1 grams of dehydrated medium were suspended in 1 liter of distilled water and brought to boiling to dissolve completely. The pH was adjusted to 7.2. The medium was then distributed in 3 – 5 ml portions into test tubes containing Craigie tubes and the preparation was then sterilized by autoclaving at 115˚c for 10 min.

2-2-3 Solid Media:-

A- Nutrient agar (Hi Media code NO M001):-

Twenty eight grams of dehydrated medium were suspended in 1 liter of distilled water and boiled to dissolve. The pH was adjusted to 7.4 and the medium was then sterilized by autoclaving at 121˚C for 15 min. Then cooled to 45- 50˚C and distributed into petri dishes in 20 ml amounts.

B- MacConkey′s agar (Hi Media code NO M081):

51.5 grams of dehydrated medium were suspended in 1litre of distilled water and boiled to dissolve. The pH was adjusted to 7.1 and media sterilized by autoclaving at 121˚C for 15 min. Then, cooled to 45- 50˚C and distributed into sterile petri dishes in about 20 ml each.

C- Sorbitol MacConkey′s agar medium:

The medium was prepared by dissolving the ingredients (see appendix) in 1L distilled water by steaming or boiling, then it was sterilized at 121˚Cfor 15 min, cooled to 45- 50˚C and distributed into sterile petri dishes in about 20 ml each.

D- Simmon's citrate agar (Difco code NO 0091-01):

24.2 grams of dehydrated medium were suspended in 1 liter of distilled water and boiled to dissolve. The pH was adjusted to 6.8. The medium was distributed into McCartney bottles and sterilized by autoclaving at 121˚C for 15 min. After sterilization the bottles were put in a slope position to solidify.

Diagnostic sensitivity test agar (DST) (Oxoide):

DST medium consist of protease peptone, veal infusion solids, dextrose, sodium chloride, di-sodium phosphate, sodium acetate, adenine sulphate, guanine hydrochloride, uracil, xanthine, and ion agar NO2.

Forty grams of the medium were suspended in one liter of distilled water, and then brought to boil to dissolve completely, sterilized by autoclaving at 121˚C for 15 minutes, then dispended into sterile petri dishes in portions of 15 ml each. The poured plates were left to solidify at room temperature on leveled surface.

2-3 Sterilization:

2-3-1 sterilization of equipments:

Petri dishes, test tubes, flasks, Pasteur pipettes and graduated pipettes were sterilized in hot air oven at 160˚C for 1 hour. Glassware such as Bijou, universal, McCartney, 125 ml size bottles were sterilized in the autoclave at 15 pounds pressures for 15 minutes at 121 ˚C.

2-3-2 Sterilization of culture media and solutions:

Nutrient agar, peptone water. MacConkey agar, Simmon's citrate agar, Malona brotht, sorbitol MacConkey agar and DST were sterilized in the autoclave at 15 pounds pressure for 15 minutes at 121˚C.

Hugh and Leifson's (OF) medium were sterilized in the autoclave at 10 pounds pressure for 10 minutes at 115˚C. Carbohydrate media were sterilized in autoclave at 115˚C for 20 minutes.

2-4 Incubation of cultures:

Cultured specimens were incubated aerobically at 37˚C over night. Next day, the culture was examined.

2-5 Identification of cultured isolates:

The colonies were identified by Gram′s-staining technique, motility test and biochemical tests, Gram′s -stain was done for the observation of the organism to confirm that they were Gram-negative bacilli. Motility of organism was examined by motility medium.

2-6- Culture Methods:

2-6-1 Primary isolation of aerobic bacteria:

-solid media:

Rectal swabs and a loopfull from each fecal sample were inoculated into sorbitol MacConkey agar, by the four-quadrant streaking methods.

- Incubation of cultures:

Inoculated media were incubated aerobically at 37˚C overnight.

- Examination of cultures:

All cultures on solid media were examined with naked eye for growth, colonial morphology and any changes in the media.

2-6-2 Sub-culturing and purification of isolates:

Apart of a typical well isolated colony was picked with a loop and streaked on the surface of fresh plate of the sorbitol MacConkey′s agar. The

process was repeated until culture become pure. The resulting growth was checked for purity by examining smears stained by Gram-stain.

All non-sorbitol fermenting colonies were inoculated into MacConkey's agar. Pure cultures were then inoculated into nutrient agar to be used for further biochemical tests.

2-7 Preparation of smears and staining method:

Smears were prepared by emulsifying part of a colony in saline and spreading it on a clean slide. The smears were then allowed to dry in air and fixed by gentle flaming, then stained by Gram-stain as described by Barrow & Feltham, 2003.

2-8 Motility Test:

The tested organisms were inoculated in tubes of motility media by stabbing the medium to a depth of 5mm, incubated at 37˚C for 24h. Motile organisms migrate through the medium which becomes turbid; growth of non-motile organisms is confined to the stab.

2-9 Biochemical methods for identification of isolated bacteria:

All biochemical testes were done according to Barrow & Feltham**,** (1993) or Cheesbrough (2000).

2-9-1- Catalase test:

This test is used to differentiate those organisms that produce the enzyme catalase from non-catalase producing organisms. The tested organism was brought into contact with 3% hydrogen peroxide (H2O2). Bubbles of oxygen are released if the organism is a catalase producer.

2-9-2- Oxidase test:

The oxidase test is used to assist in the identification of bacteria which produce the enzyme cytochrome oxidase. The test was conducted by using a piece of filter paper soaked with a few drops of oxidase reagent (tetra-methyle-p-phenylene diamine dihydrochloride). A colony of the tested organism was then smeared on the filter paper. If the organism is oxidase producing, the phenylenediamine in the reagent will be oxidized to deep purple color.

2-9-3- Oxidation fermentation of glucose:

To find out whether the organism attack carbohydrate by oxidation or fermentation, the OF test is made by growing the tested organism in two

tubes of Hugh & Leifson's medium; the medium in one tube was covered with a layer of soft paraffin, the tubes were incubated and examined daily for 14 days. Oxidizers show acid production in the open tube only (yellow color) while fermenters show acid in the paraffin-covered tube and open tube.

2-9-4-Fermentation of sugars:

The test cultures were inoculated into peptone water sugar medium, then incubated at 37˚C and examined daily for up to 7 days. Acid production was indicated by appearance of a reddish color, whereas gas production was indicated by development of an empty space in the Durham's tube.

2-9-5- Indole test:

Testing for indole production is important for identification of bacteria which breakdown the amino acid tryptophan with the release of indole. The tested organism was cultured in peptone water and incubated at 37˚C for 48h. Indole production was detected by Kovac's reagent which contains 4(P)-dimethylamino benzaldehyde, reacts with indole to produce a red coloured compound.

2-9-6- Methyl red (MR) test and Voges-Proskauer (VP) test:

For detection acetyl methyl carbinol or acetoin, maybe carried out on the same tube of culture.

The glucose phosphate medium was inoculated by the tested organism and incubated at 37˚C for 2 days. 2 drops of methyl red solution were added, shaked and examined:

Red colour = +ve

Orange= ± ve

Yellow= - ve

After reading the MR reaction the same culture can be used for the VP test, 0.6 ml 5% α-naphthol solution and 0.2 of 40% KOH aq.soln were added to the culture, shaked, the tube was slopped and examined after 15 min and 1h.

A positive reaction is indicated by a strong red color.

2-9-7- Citrate utilization test:

The test is based on the ability of an organism to use citrate as its only source of carbon.

Simmon's citrate medium was inoculated with a suspension of the tested organisms, incubated at 37˚Cand examined daily for 14 days. Blue color or turbid bottles were considered positive. Organisms which did not utilize citrate failed to produce any change in the medium.

2-9-8- Malonate utilization test:

Malonate broth medium was lightly inoculated and incubated at 37˚C for 24h. A positive reaction was indicated by a deep blue color.

2-9-9- Sorbitol fermentation test:

After addition of sorbitol and indicator (Andrades) to the peptone water the medium was inoculated by the tested culture and incubated at 37˚C and examined daily for 7 days. Positive reaction was indicated by change in color to red.

2-10- Antibiotic Sensitivity Test:

Sensitivity of some isolates of *Escherichia coli* to 12 antimicrobial agents was determined by the standard disc diffusion method. The antimicrobial agents used, their abbreviation, concentration and origin has been shown in Table (1) below. The media used for sensitivity test was Diagnostic Sensitivity Test agar (DST) medium.

A single colony of the tested organism was inoculated into 5 ml nutrient broth medium and incubated for 2h at 37˚C. Using sterile micro-pipette 1-2 ml of broth culture were added to DST medium, the surface of the medium was completely covered by the tested broth culture and the excess broth was aspirated and the plates were allowed to dry for 15-30 min., then , sterile forceps were used to apply the discs over the surface of the medium and the plates were incubated at 37˚C for 24 h. The diameter of the zone of inhibition to each antibiotic against each tested isolate was measured and the results were recorded. The obtained results were interpreted according to the zone diameter interpretive chart for MRCOMEX antimicrobial test discs.

Table (1): The abbreviations and concentrations of Antimicrobial agents used for characterization of *E. coli* isolates.

Antimicrobial agent	Abbreviation *	Concentration (µg)
Ampicillin\Sulbactam	AS	20 mcg
Co-Trimoxazole	BA	25mcg
Cefotaxime	CF	30 mcg
Piperacillin/Tazobactam	TZP	100/10 mcg
Chloramphennicol	CH	30 mcg
Ciprofloxacin	CP	5 mcg
Ceftizoxime	CI	30 mcg
Tetracycline	TE	30 mcg
Ofloxacin	OF	5 mcg
Gentamicin	GM	10 mcg
Amikacin	AK	30 mcg
Pefloxacin	PF	5 mcg

* Abbreviation aren't derived from the name of the antibiotic but coded by the producing company.

** All above mentioned antimicrobial agents are manufactured by Axiom Company (India).

CHAPTER Three

RESULTS

3-1 Examination of fecal samples for detection of sorbitol fermenter (SF) and non-sorbitol fermenter(NSF) *Escherichia coli*:-

A total of 45 fecal samples were collected from healthy domestic animals (cattle, sheep and goats) from Shambat, Alkalakla and Kuku localities in Khartoum state.

3-1-1 Isolation and colonial morphology:-

The colonial morphology was studied on:

- Sorbitol MacConkey agar:

Colourless colonies (indicating failure to ferment sorbitol) and red colonies (sorbitol fermenters) on this medium were observed after overnight incubation at 37˚C aerobically.

- MacConkey′s agar:-

A luxuriant growth was produced by all isolates on MacConkey′s agar following overnight aerobic incubation, variable colony sizes with entire or irregular edges were obtained, and dark pink colonies on this medium were produced by isolates indicating fermentation of lactose.

According to the primary isolation on sorbitol MacConkey agar and sub-cultured of non-sorbitol fermenter (NSF) on MacConkey agar, out of 47 isolates were obtained; 39 isolates sorbitol fermenters (SF) and 8 isolates non-sorbitol fermenters (NSF) and lactose fermenters.

3-1-2 Microscopic characterizations of isolates:-

All isolates examined under the microscope revealed Gram-negative short rods, occurring singly or in pairs.

3-2 Examination of rectal swabs for detection of SF and NSF *Escherichia coli*:-

A total of 60 rectal swabs were collected from previous animals in addition to chickens from previous areas. According to the primary isolation on sorbitol MacConkey agar and sub-cultured of non-sorbitol ferminter (NSF) on MacConkey′s agar, out of 55 isolates obtained 49 isolates were (SF) and 6 isolates were (NSF) and lactose fermenter. All isolates examined under microscope revealed Gram-negative short rods, occurring singly or in pairs.

3-3 The biochemical reactions of the isolates:-

(SF) and (NSF) isolates were subjected to primary and secondary tests. The results of motility test for (SF) revealed 65 motile isolates and 23 non motile. Nine of (NSF) isolates from NSF were motile while 5 isolates were non motile.

Any isolates that reacted negatively to oxidase test,VP test, citrate utilization test, malonate utilization test, and positively to catalase test, MR test, indole test, sorbitol test was considered classical *E. coli*, and if sorbitol-negative was considered E. coli serotype O157:H7 Table (2).

According to biochemical tests (8) isolates from fecal samples were likely to be *E. coli* serotype O157:H7 (non sorbitol fermenter) and (37) isolates were likely to be other classical *E. coli* (sorbitol ferminter).

As shown in Table (3), *E. coli* O157:H7 isolates were (2) from cattle, (3) from sheep and (3) from goats. The total number of other sorbitol ferminting *E.coli* isolated from faecal samples were (37); (11) from cattle, (12) from sheep and (14) from goats.

Five isolates from rectal swabs were likely to be E. coli serotype O157:H7 and (48) isolate were identified as other *E.coli* strains.

As shown in Table (4), *E.coli* O157:H7 isolates are distributed as follows: (1) from cattle, (1) from sheep, (2) from goats and (1) from poultry, the total number was (5) isolates. The total number of sorbitol ferminting *E.coli* isolated from rectal swabs was (48). (12) from cattle; (12) from sheep; (9) from goats and (15) from poultry. The total number of *E. coli* O157:H7 isolates obtained from both fecal (45 samples) and rectal swabs (60 samples) were 13 (12.3%) Table (5)

3-4 Sensitivity Test:-

The result of antimicrobial susceptibility of all *E. coli* isolates (SF & NSF) from fecal samples and one (NSF) isolate from chicken rectal swab revealed that there was high resistance to Ampicillin/Sulbactam.

Most (91.4%) of (SF) *E. coli* strains were sensitive to Chloramphenicol and 89.3% isolates were sensitive to Gentamicin, while 88.8% of (NSF) *E. coli* isolates were sensitive to Chloramphenicol and 66.6% sensitive to Gentamicin. Both SF & NSF isolates showed variable susceptibility to different antibiotics as shown in Table (6) and (7).

All sorbitol fermenting *E. coli* ifolated from cattle (100%) were sensitive to Chloramphenicol and Ciprofloxacin at concentration 5 mcg and Pefloxacin, while (NSF) *E. coli* isolates (100%) from cattle were sensitive to Pefloxacin only.

Most (SF) *E. coli* sheep tested isolates (90%) were sensitive to Chloramphenicol and Pefloxacin, while 100% of (NSF) *E. coli* isolated from sheep was sensitive to Co-Trimoxazole, Chloramphenicol, Ciprofloxacin and Pefloxacin.

All (SF) *E. coli* isolated from goat (100%) were sensitive to Chloramphenicol and Ciprofloxacin. While all (NSF) *E. coli* isolated from goat (100%) were sensitive to Chloramphenicol and Gentamicin.

Most (SF) *E. coli* isolated from poultry (83%) were sensitive to Gentamicin, while all (NSF) *E. coli* tested isolates (100%) of poultry were sensitive to Piperacillin/Tazpbactam, Chloramphrnicol, Ciprofloxacin, Gentamicin and Amikacin. All tested isolates of poultry either from (SF) *E. coli* or (NSF) *E. coli* were resistance to Tetracycline.

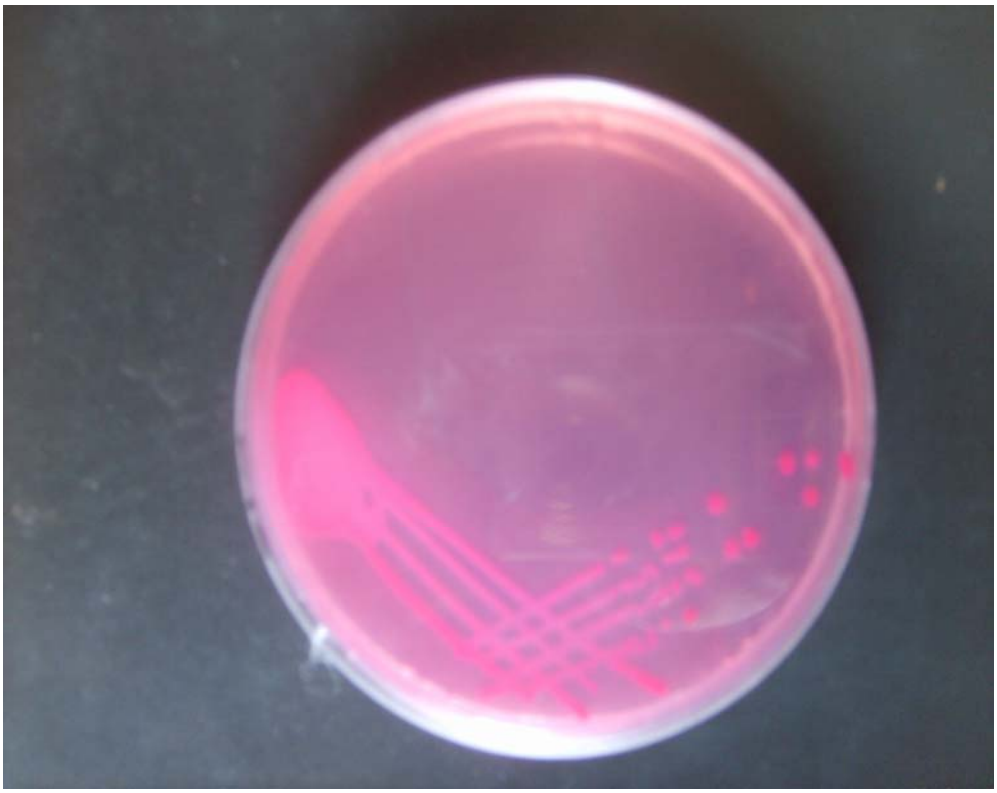

Figure (1-a): *E. coli* O157:H7 on MacConkey's agar. Note the dark pinkish colors.

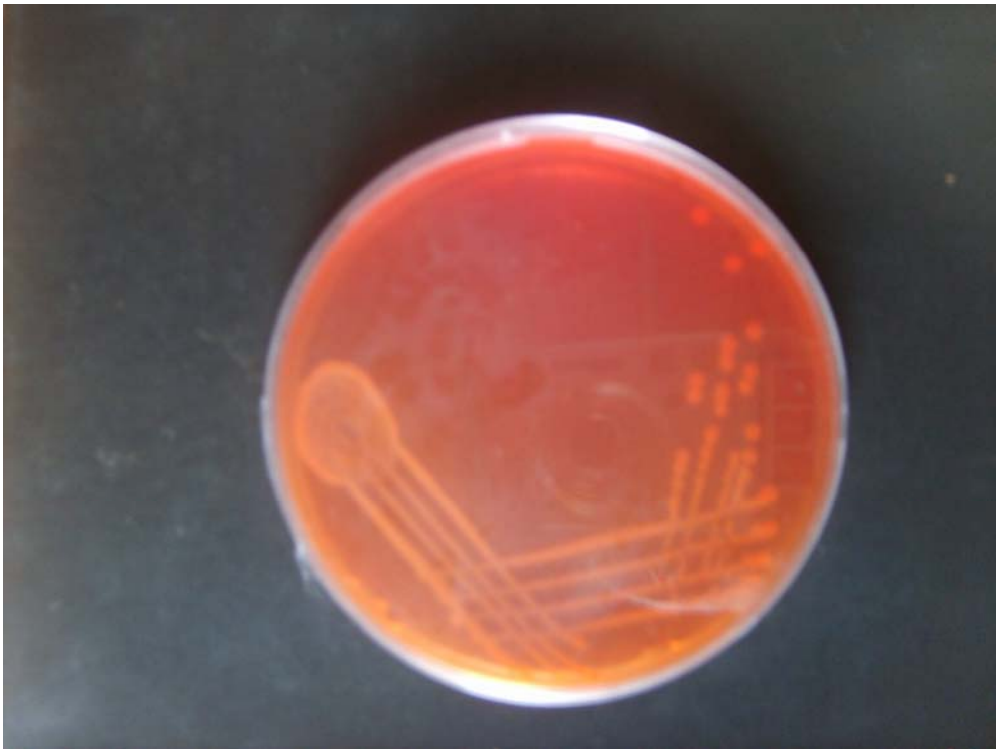

Figure (1-b) *E. coli* O157:H7 on Sorbitol MacConkey's agar. Note colorless colonies.

Table (2) Some Biochemical reactions of *E.coli* O157:H7 and other classical *E.coli* isolates:

Test	*E.coli* O157:H7	Other *E.coli*
Motility	d	d
Growth in SMAC	Colourless colonies	Dark pink colonies
Growth in MAC	Dark pink colonies	Dark pink colonies
Oxidase	_	_
Catalase	+	+
Oxidation Fermentation(OF)	F	F
Fermentation of glucose	+g	+g
Indole	+	+
MR test	+	+
VP test	_	_
Citrate utilization	_	_
Malonate utilization	_	_
Sorbitol	_	+

d: different (some isolates were motile and some were non-motile)

SMAC: sorbitol MacConkey's agar.

MAC: MacConkey's agar.

+g: fermentation of glucose with gas production.

F: fermentative.

+: Positive.

_: Negative.

Table (3) shows the number of *E.coli* O157:H7 (non-sorbitol fermenter) and other *E.coli* strains (sorbitol fermenter) isolated from fecal samples.

Number and sources of samples	(SF) *E.coli*	(NSF) *E.coli*	Total of isolates
Cattle: 15	11 (73.3%)	2 (13.3%)	13
Sheep: 15	12 (80%)	3 (20%)	15
Goat: 15	14 (93.3%)	3 (20%)	17
Total: 45	37 (82.2%)	8 (17.7%)	45

Table (4) the number of *E.coli* O157:H7 and other *E.coli* strains isolated from rectal swabs.

Number and sources of samples	(SF) *E.coli*	(NSF) *E.coli*	Total of isolates
Cattle: 15	12 (80%)	1 (6.6%)	13
Sheep: 15	12 (80%)	1 (6.6%)	13
Goat: 15	9 (60%)	2 (13.3%)	11
Poultry: 15	15 (93.7%)	1 (6.6%).	16
Total: 60	48 (80%)	5 (9.4%)	53

- **SF: sorbitol fermenting**.
- **NSF: non sorbitol fermenting**.

Table (5) shows the recovery rates of *E. coli* O157:H7 isolated from fecal samples and rectal swabs from different animal species:

Type of sample	Number of sample examined	Number of isolates recovered	Recovery percentage
Fecal samples	45	8	17.7%
Rectal swabs samples	60	5	8.3%
Total	105	13	12.3%

Table (6): Antibiotics sensitivity of 47 isolates (SF) *E. coli* isolated from cattle, sheep, goats and poultry:

Antibiotic	No. of sensitive isolates (% age)	No. of intermediate isolates (% age)	No. of resistant isolates (% age)
Ampicillin/Sulbactam (AS) 20 mcg	0 (0%)	0 (0%)	47 (100%)
Co-Trimoxazole (BA) 25 mcg	34 (72.3%)	0 (0%)	13 (27.6%)
Cefotaxime (CF) 30 mcg	0 (0%)	30 (63.8%)	17 (36.1)
Piperacillin/Tazobactam (TZP) 100/10 mcg	14 (29.7%)	20 (42.5%)	13 (27.6%)
Chloramphenicol (CH) 30 mcg	43 (91.7%)	2 (4.2%)	2 (4.2%)
Ciprofloxacin (CP) 5 mcg	35 (74.4%)	6 (12.7%)	6 (12.7%)
Ceftizoxime (CI) 30 mcg	22 (46.8%)	24 (51.1%)	1 (2.1%)
Tetracycline (TE) 30 mcg	4 (8.5%)	10 (21.2%)	33 (70.2%)
Ofloxacin (OF) 5 mcg	11 (23.4%)	26 (55.3%)	10 (21.2%)
Gentamicin (CM) 10 mcg	42 (89.3%)	4 (8.5%)	1 (2.1%)
Amikacin (AK) 30 mcg	29 (61.7%)	15 (31.9%)	3 (6.3%)
Pefloxacin (PF) 5 mcg	36 (76.5%)	0 (0%)	11 (23.4%)

Table (7): sensitivity of (NSF) 9 *E. coli* isolated from cattle, sheep, goats and poultry to various antimicrobial agents

Antimicrobial agents	No. of sensitive isolates (% age)	No. of isolates intermediate isolates (% age)	No. of resistant isolates (% age)
Ampicillin/Sulbactam (AS) 20 mcg	0 (0%)	0 (0%)	9 (100%)
Co-Trimoxazole (BA) 25 mcg	5 (55.5%)	0 (0%)	4 (44.4%)
Cefotaxime (CF) 30 mcg	1 (11.1%)	7 (77.7%)	1 (11.1%)
Piperacillin/Tazobactam (TZP) 100/10 mcg	4 (44.4%)	4 (44.4)	1 (11.1%)
Chloramphenicol (CH) 30 mcg	8 (88.8%)	0 (0%)	1 (11.1%)
Ciprofloxacin (CP) 5 mcg	4 (44.4%)	3 (33.3%)	2 (22.2%)
Ceftizoxime (CI) 30 mcg	5 (55.5%)	4 (44.4%)	0 (0%)
Tetracycline (TE) 30 mcg	0 (0%)	5 (55.5%)	4 (44.4%)
Ofloxacin (OF) 5 mcg	2 (22.2%)	4 (44.4%)	3 (33.3%)
Gentamicin (GM) 10 mcg	6 (66.6%)	3 (33.3%)	O (0%)
Amikacin (AK) 30 mcg	4 (44.4%)	5 (55.5%)	0 (0%)
Pefloxacin (PF) 5 mcg	7 (77.7%)	0 (0%)	2 (22.2%)

CHAPTER Four

Discussion

The present study was designed and conducted to investigate the distribution of *Escherichia coli* serotype O157:H7 among the other enteric *E. coli* serotype inhabiting the intestine of healthy domestic animals. Four species were sampled; namely cattle, sheep, goats and chickens. The samples were collected from Shambat, Alkalakla and Kuku localities. In this study sorbitol MacConkey's agar was used as a primary isolation medium, two different sampling techniques (fecal sampling and rectal swabbing were included).

The results of 45 fecal samples examined from the different species revealed the presence of classical *E. coli* in 37 (82%) samples and *E. coli* O157:H7 in 8 (17.7%) samples; 2 (13.3%) from cattle, 3 (20%) from sheep and 3 (20%) from goats. This finding supported that of Beutin*, et al*., (1993) who isolated this organism from fecal samples of healthy domestic animals fecal samples including cattle, sheep, goats, cats, dogs and pigs.

The result of 60 rectal swabs examination from four different animal species showed the isolation of classical *E. coli* from 48 (80%) samples and *E. coli* O157:H7 from 5 (9.4%) samples; 1 (6.66%) from cattle, 1 (6.66%) from sheep, 2(13.3%) from goats and 1(6.66%) from chicken. this is almost comparable to result obtained by Beutin *et al.,* (1993) who was not succeeded to get any isolate of *E. coli* O157:H7 from chickens, but in agreement of Beery *et al*., (1985) who reported the ability of this organism to colonized chickens cecae, and this indicates that chickens may serve as reservoirs for *E. coli* O157:H7.

The isolation of *E .coli* O157:H7 from cattle all over the world has since identified cattle as principal reservoir of *E. coli* O157:H7 (Montenegro, *et al*., 1990 and Wells, *et al*., 1991). Very little is known about the occurrence of *E. coli* O157:H7 in other species of domestic animals. The presence of *E.coi* O157:H7 in sheep and goats indicates that these, together with cattle, represent an important natural reservoirs, and *E. coli* O157:H7 appear to be present in high number in feces from animals without any signs of disease.

The frequency of *E. coli* O157:H7 among other classical *E. coli* in fecal sample was 17.7% compared to 9.4% rectal swabs. This proved that *E. coli* O157:H7 yielded more colonies in culture directly from direct fecal samples than cultures from rectal swabs, this finding do not agree with the finding of Davis *et al.,*2006 who reported that recto-anal mucosal swab culture was as sensitive as fecal culture.

The presence of *E. coli* O157:H7 is affect by the rates of epithelial cell proliferation in lower gastrointestinal tract, Bernadene, *et al*., (2000) reported that cattle of slower rates of intestinal cell proliferation in cecum and the distal colon were cultured positive for *E. coli* O157:H7 than cattle with faster cell proliferation rates. This report interprets why few rectal swabs cultured positive.

We have collected samples from only a relatively small geographical area, but we have no reason to believe that our finding cannot be generalized. This view is supported by the few report on isolation of *E. coli* O157:H7 from sheep, cats and goat milk (Abass *et al.,* 1989, Dorn *et al*., 1989 and Samadpour *et al*., 1990).

MacConkey's sorbitol bile salt agar proved to be a satisfactory medium for primary isolation of *E. coli* serotype O157:H7 and this agree with the finding of Wells *et al*., (1983) and March and Ratnam (1986).

The results of sensitivity test of non sorbitol fermenter *E. coli* and sorbitol fermenter *E. coli* isolates showed that they have more than one pattern of susceptibility to the 12 antibiotics. All isolates showed high resistance to Ampicillin/sulbactam at 20 mcg concentration. This supported the fact that Gram-negative bacteria, including enterobacteriaceae produce β-lactamases, enzymes which interfere with the action of beta-lactam ring in some antibiotics possessing this ring like Ampicillin.

Also non sorbitol fermenting *E. coli* and sorbitol fermenter agree with others in their sensitivity to Chloramphenicol and Gentamicin and Pefloxacin in different degrees. Also there was lower sensitivity to Tetracycline. This finding supported studies on the susceptibility of *E. coli* isolated from healthy and diseased animals to different antibiotics (Eiman, 1998 and Mohammed,2006), who reported high sensitivity to Gentamicin, and high prevalence of resistance to Tetracycline observed in *E. coli*. The resistance to

Tetracycline can be attributed to the extensive uses of this antibiotic among farmers.

Conclusion:-

The following is the out put of this study:-

- The present investigation has thrown some light on the incidence of *Escherichia coli* O157:H7 isolated from healthy animals and its revealed (12.3%) *E. coli* O157:H7 in healthy animals.
- *E.coli* O157:H7 found to be particularly frequent in three ruminant species (cattle, sheep and goats) and *E.coli* O157:H7 was much more sporadically isolated from nonruminants (chickens).
- Sorbitol MacConkey´s agar medium is a useful, rapid and reliable screening aid for the detection of *E. coli* O157:H7 in fecal specimens.

Recommendation:-

- It is suggested that further studies should be carried out to investigate other possible sources of *Escherichia coli* O157:H7 and to link these sources with possible epidemics in human in all over Sudan.
- Molecular detection of specific genes of *E. coli* serotype O157:H7 in feces of domestic animals either direct or after enrichment may be useful in epidemiology of human infection.
- *E. coli*, including serotype O157:H7 was found sensitive to a number of antibiotics notably chloramphenicol and gentamicin.
- Further extensive work should be carried out to survey the prevalence of antibacterial drug-resistance to determine the most effective antibiotic.

References

Abass, S., A. Franklin, I. kuehn, F. Qrskov, and I. Qrskov. (1989). Cytotoxin activity on Vero cells among *Escherichia coli* strains associated with diarrhea in cats. Am. J. Vet. Res. **50**:1294-1296.

Armstrong, G.L.,J.Hollings Wrth and G. Morris,Jr. (1996). Emerging food borne pathogens: *Escherichia coli* O157:H7 as a model of a new pathogen into theFood supply of developed world. Epedemiol Rev.**18**:29-51.

Ascheson, D.W.K, R.Moore, S.De Breucker, L.L.Lincicome, M.Jacewicz, E.Skutelsky, and G.T.Keusch. (1992). Translocation of Shiga toxin polarized intestinal cell in tissue culture. Infect. Immun. **64**:3294-3300.

Barrow,G.I and Feltham R.K.A. (1993). Cowan and Steel's Manual of Identification of Medical Bacteria, 3rd edition. Cambridge University press.

Beery J. T, Doyle M. P, and Schoen J. L, (1985). Colonization of chicken cacae by *Escherichia coli* associated with hemorrhagic colitis. Appl. Environ. Microbiol. **49**:310-315.

Benjamin,M.M., and A.R.Datta. (1995). Acid tolerance of enter- hemorrhagic *Escherichia coli*. Appl. Environ. Microbiol. **61**:1669- 1672.

Bernadene A. Magnuson, Margaret Davis, Suzanna Hubele, Paula R. Austin. Indira T. Kudva, Christopher J. Williams, Carl W. Hunt, and Carolyn J. Hovde. (2000). Rumminant gastrointestinal cell proliferation and of *Escherichia coli* O157:H7. Infect and Immun. **68**(7):3808-3814.

Besser,R.E.,S.M.Lett, J.T.Weber, M.P.Doyle, T.J.Barrett, J.G.Wells, and P.M.Griffin. (1993). An outbreak of diarrhea and hemolytic uremic syndrome from Escherichia *coli* O157:H7 in fresh pressed apple cider. JAMA **269**:2217- 2220.

Beutin,L., D.Geier, H.Steinrück, S.Zimmermann, and F.Sheutz. (1993). Prevalence and some properties of (shiga- Like toxin)-producing *Escherichia coli* in seven Different species of healthy domestic animal. J. Clin. Microbiol **31**:2483- 2488.

Beutin,L., S.ALeksic, S.Zimmermann and K.Gleier. (1994) Virulence factors and phenotypic traits of verotoxigenic strain of *Escherichia coli* isolated from human patients in Germany. Med. Microbiol. Immunol (Berlin). **183**:13-21.

Bergey,D. H and J. G.Holt.(1994). Bergey's Manual of Determinative Bacteriology. 9th edition Springer Publisher p 175- 183.

Bogaard. A.E, London.N, Driessen. C, and Stobberingh. E. E. (2000). Antibiotic resistance of fecal Escherichia coli in poultry, poultry farms and poultry slaughters. J. Anti.Chemother. **47**:763-771.

Brunder,W., H.,Schmidt, and H.Karch. (1996). Katp, a novel catalase-peroxidase encoded by the large plasmid of enterohemorrhagic *Escherichia coli* O157:H7. Microbiology **142**:3305-3315.

Buxton. A and Frazer. G. (1977). Animal microbiology. Vol 1. 1st ed Black well Scientific Publications. Oxford, London, Edinburgh and Melbourne.

Carlton L. G. (2004). Pathogenesis of bacterial Infections in Animals. 3rd ed. Black Well publisher p 212- 213.

Carter, G.R. (1986). Essentials of Veterinary Bacteriology and Mycology. 3rd ed. Lea&Febiger Philadelphia publisher. p145.

Cheesbrough, M. (2002). District Laboratory Practice in Tropical Countries part 2. Cambridge University press.

Center for Disease Control Prevetion (1995) *Escherichia coli* O157:H7 outbreak linked to commercially distributed drycured salami-Washington and California, 1994. Morbid Mortal . weekly Rep.**44**:157- 160.

Center of Disease Control and Prevention. (2006) Question &answers: sickness caused by E. coli. http://www.cdc.gov/ecoli/qa-ecoli-sicknes.htm.

David. G. W, Charlene. H, John. J. M, Sherry. A, Shaohua. Z, Margie. D. L, Lance. B, Thomas. F and Julie. S. (2000). Characterization of Chloramphenicol and Florfenicol resistance in Escherichia coli associated with bovine diarrhea. J. Clin. Microb. **38**(12):4593-4598.

Davis.M.A, Daniel H. Rice, Haiqing Sheng, Dale D. Hancok, Thomas E. Besser, Rowland Cobbold, and Carlyon J,H. (2006). Comparison of cultures recto-anal junction mucosal swabs and feces for detection

of *E. coli*O157:H7 in dairy heifers. Appl and Environ Microbiol **72**(5):3766-3770.

Dorn, c. R., S. M. Scotland, H. R. Smith, G. A. Willshaw, and B. Rowe. (1989). Properties of vero cytotoxin-producing *Escherichia coli* of human and animal origin belonging to serotypes other than O157:H7. Epidemiol. Infect. **103**:83-95.

Dosogne,H., F.Vangroen Weghe, and C.Burvenich (2002) Potential mechanism of action of J5 vaccine in protection against severe bovine coliform mastitis. Vet. Res **33**:1- 12.

Douglas,F.Fix. 2007.Microbiology Principles and Applications. Department of Microbiology southern Illinois University at Carbondale .http://www.cehs.siu. Edu/fix/medmicro/pix/classic.

Eiman A O. (1998). Isolation and characterization of the genus Escherichia from animal and human. M. Sc. Thesis. University of Khartoum.

Farmer,J.J., III, and B.R.Daris. (1985). H7 antiserum- sorbitol fermentation medium: a single- tube screening method for detecting *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol. **22**:620- 625.

Farmer,J.J, III, (1995). Eenterobacteriaceae: introduction and Identification, p. 438-449 In P.R.Murray, E.J.Baron, M.A.Pfaller, F.C.Tenover and R.H.Yolken (ed), Manual of Clinical Microbiology. 6th ed. ASM press Washington D.S. In J.P.Nataro and J.B.Kaper (1998) .Diarrheagenic *Escherichia coli*. Clin. Microbiol Rev **11**:142- 201.

Fegan,N.,Desmarcheluer, P. (1999). Shiga toxin- producing *Escherichia coli* in sheep and preslaughter lamb in eastern Australia. Letter in Applied Microbiology.**28**(5):335- 339.

Fratamico,P.M., S.Bhaduri, and R.L.Buchanan. (1993). Studies on *Escherichia coli* serotype O157:H7 strains containing a60-MDa plasmid and on 60-MDa plasmid- cured derivatives. J.Med. Microbiol. **39**:371-381.

George,W.Beran. (1994). Hand book of Zoonoses section A Bacterial, Rickettial, Chlamydial, and Mycotic. 2nd CRD press p. 333- 336.

Griffin,P.M., L.C.Olmstead and R.E.Petras. (1990). *Escherichia coli* O157:H7 associated colitis: and a clinical and histological study of 11 cases. Gastroenterology **99**:142- 149.

Griffin,P.M., and R.V.Tauxe. (1991)The epidemiology of infection caused by *Escherichia coli* O157:H7 other enterohemorrhagic *E. coli*, and the associatd hemolytic uremic syndrome. Epidemol. R. **13**:60- 98.

Griffin,P.M.,. (1995). *Escherichia coli* O157:H7 and other enterohemorrhagic *E. coli* p. 739- 761 In M.J.Blaser, P.D.Smith, J.I.Ravadin, H.B.Greenberh, and R.L. Guerrant (ed) Infections of the gastrointestinal tract Raven prees, New York. N.Y. In J.P.Nataro and J.B. Kaper. (1998). Diarrheagenic *Escherichia coli*. Clin. Microbial. Rev **11**:142- 201.

Hagan.W.A and Timoney.J.F. (1988). Hagan and Bruner's Microbiology and Infectious Diseases of Domestic Animals. 8th ed. Cornell University press p. 61.

Harrigan W.F. (1997). Laboratory Methods in Food Microbiology 3rd ed Academic press.

Hill, A.W. (1994). *Escherichia coli* mastitis In C.L.Gyles (ed) Pathogenesis of Bacterial Infection in Animals 3rd ed p 117- 133.

Hirsh.D.C., and Yuanchang. Z. (1999). Veterinary Microbiolog Black well science publisher.

Imberechts, H., p.Deprez, E.Van.Drissche, and P.Pohl. (1997a). Chicken egg yolk antibodies against F18ab fimbriae of *Echerichias coli* inhibit shedding of F18 positive E. coli By experimental infected pigs. Vet. Microbiol. **54**:328- 341. In C.L.Gyles (ed) 2004 Pathogenesis of Bacterial Infections in Animals. 3rd ed . Back well publishing.

James C.Paton, and Adrienne W.Paton. (1998). Pathogenesis and diagnosis of shiga toxin-producing *Escherichia coli* infection. Clin. Microbiol. Rev. **11**:450- 479.

Jarvis,K.G.,J.A.Giron,A.E.Jerse,T.K.McDaniel,M.S.Donnenberg, and J.B.Kaper. (1995). Enteropathogenic *Escherichia coli* contain a specialized secretion system necessary for the export of proteins involved in attaching and effacing lesion formation. Proc.Natl. Acad.Sci. USA **92**:450- 479.

Joan L. Slonczewski, and John W.Foster. 2007. Genus *Esherichia coli* . http://microbiology.kenyon.edu/.

Johnson,R.P., R.C.Clarke, J.B.Wilson, S.C.Read, K.Rahn, S.A. Renwick, K.A. Sandhu, D.Alves, M.A.Karmali, H.Lior, S.A.McEwen,J.S.Spika, and C.L.Gyles. (1993). Growing concerns and recent outbreaks involving non-O157:H7 serotypes of verotoxigenic *Escherichia coli* . J. Food. Prot. **59**:1112-1122.

Kandel,G., A.Donohue-Rolfe,M.Donowitz, and G.T.Keusch.(1989). Pathogenesis of *Shigella* diarrhea XV1 selective targettinge of shiga toxin to villus cells of jejunum explains the effect of the toxin on intestinal electrolytes transport . J.Clin.Invest. **84**:1509- 1517.

Karch, H., D.I.Tarr, and M.Bieleszewska. (2005). Enteroheamorrhagic *Escherichia coli* in human Medicine. Int.J.Med.Microbiol. **295**:405-418.

Karmali,M.A. (1989). Infection by verocytotoxin-producing *Escherichia coli*. Clin.Microbiol.Rev. **2**:15-38.

Karmali,M.A., B.T.Steele, M.Petric, and C.Lim. (1983). Sporadic cases of haemolytic uremia syndrome associated with fecal cytotoxin and cytotoxin producing *Escherichia coli* in stool. Lancet **i**:619-620. In J.P.Nataro and J.B Kaper. (1998). Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. **11**(1):142-201.

Keenan,K.P.,D.D.Sharpnack, H.Collins, S.B.Formal, and A.D. O'Brien. (1986). Morphological evaluation of the effects of shiga toxin and *Escherichia coli* shiga-like toxin on the rabbit intestine. Am.J.Pathol. **125**:69-80.

Kibel,M.A., and P.J.Barnard. (1968). The heamolytic uremic syndrome: a survey in South Africa. S.Afr Med.j. **42**:692-698. In J.P.Nataro and J.B. Kaper. (1998) Diarrheagenic *Escherichia coli*. Clin.Microbiol.Rev **11**:142-201.

Konowalchuk,J.I.Speirs, and S.Stsvric. (1977). Vero- response to a cytotoxin of *Escherichia coli*. Infect.Immun. **18**:775-779.

Law,D., and J.Kelly. (1995). Use of heme and hemoglobin by *Escherichia coli* O157:H7 and shiga- like toxin- producing *E. coli* serogroups. Infect.Immun. **63**:700-702.

Levine,M.M., J.Xu, J.B.Kaper, H.Lior, V.Prado, B.Tall, J.Nataro, H.Karch,and K.Wachsmuth. (1987). A DNA probe to identify enterohemorrhagic *Escherichia coli* O157:H7 and other serotypes that cause hemorrhagic colitis and hemolytic uremic syndrome .J.Infect.Dis. **156**:175- 182.

Lie,Z., C.Bell,A.Buret, R.Robins-Browne, D.Stiel, and E.O'Loughlin (1993). The effect of enterohemorrhagic *Escherichia coli* O157:H7 on intetinal structure and solute transport in rabbit. Gastroenterlogy. **104**:467-474.

Lydia. B, Cynthia. A. L, Margie. D. I, Anne. O. S, David.G. W, Stephan. G. T, and John.J.M. (1999). Incidence and characterization of intergrons, genetic elements mediating multiple-drug resistance, in avian Escherichia coli. Antimicrobiol. Agent. Chemoth. **43**(12):2925-2929.

MacFaddin, Jean F. (1980). Biochemical tests for identification of medical bacteria. William&Wikins, p 441.In *htt://www. en.wikipedia.org/wilki/*.

Madigan M, and Martinko J. (2005) Brock biology of microorganisms, 11th ed, Prentice Hall In *htt://www. en.wikipedia.org/wilki/*.

March, S.B., and S.Rantnam. (1986). Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. Clin.Microbiol. **23**:869-862.

McKee, M.L., and A. D.O'Brien. (1995). Investigation of enterohemorhagic *Escherichia coli* O157:H7 adherence characteristic and invasion potential reveals new attachment pattern shared by intestinal *E. coli*. Infect.Immun. **63**:2070-2074.

McCarthy, M**.** 1996. *E. coli* O157:H7 outbreak in USA traced to apple juice. Lancet **348**:1299. In J.P.Nataro and J.B Kaper. (1998). *Diarrheagenic Escherichia coli*. Clin. Microbial.Rev. **11**:142-201.

McDonough.P.L, C.A.Rossiter, B.Rebhun, Susan.M.Stehman, Donald.Lein, and Sang.J.Shin. (2000). Prevelance of *Escherichia coli* O157:H7from Cull Dairy Cows in New York State and Comparison of Culture Methods used during preharvest food safety investigation. J. Clin. Microbial. **38**:(1)318-322.

Mills, M., and S.M.Pyne. (1995). Genetic and regulation of heme iron transport in *Shigella dysenteriae* and detection of an analogous system in *Escherichia coli* O157:H7. J. Bacteriol. **177**:3004- 3009.

Mohammed. K. M. (2006). Anti biotic sensitivity of Escherichia coli isolatedfrom animals and human sources. M. Sc. Thesis. University of Khartoum.

Montenegro, M. A., M. Bülte, T. Trumpf, S. Aleksic, G. Reuter, E. Bulling, and R. Helmuth. (1990). Detection and characterization of fecal verotoxin-producing *Escherichia coli* from healthy cattle. J. Clin. Microbiol. **28**:1417-1421.

Morgan,G.M., Newman, S.R.Palmer, J.B.Allen, W.Shepared, A.M.Rampling, R.E.Warren, R.J.Gross, S.M.Scotland, and H.R.Smith. (1988). First recognized community outbreak of haemorrhagic colitis due to verotoxin- producing *Escherichia coli* o157:H7 in the UK. Epidemiol. Infect. **101**:83-91.

Nataro. J. P and Kaper. J. B. (1998). Diarrheagenic *Escherichia coli*. Clin. Microbiol.Rev. **11**:142- 201.

O'Brien,A.D., G.D.Laveck, M.R.Thompson, and S.B.Formal. (1982). Production of *Shigella dysenteriae* type 1-like cytotoxin by *Escherichia coli*. J. Infect. Dis. **146**:765- 769.

O'Brien,A.D., T.A.Lively, ME.Chen, S.W.Rothman and S.B. Formal. (1983). *Escherichia coli* O157:H7 strains associated with haemorrhagic colitis in the United State producing a *Shigella dysenteriae*1 (shiga) like cytotoxin. Lancet **i** :702 . In J.P.Naarto and J.B.Kaper. (1998). Diarrheagenic *Eschhericia coli.* Clin. Microbial. Rev. **11**:142-201.

O'Brien,A.D., J.W.Newland, S.F.Miller, R.K.Holmes, H.W.Smit and S.B.Formal. (1984). Shiga-like toxin converting Phages from *Escherichia coli* strains that cause Haemorrhagic colitis or infantile diarrhea. Science. **226**:694-696.

O'Brien,A.D., and R.K.Holmes. (1987). Shiga-like toxins. Microbiol Rev. **51**:206- 220.

O'Brien,A.D., V.L.Tesh, A.Donhue-Rolfe, M.P.Jackson, S.Olsne K.Sandvig, A.A.Lindberg and G.T.Keusch. (1992). shiga-toxin:biochemistry,

genetic, mode of action and role in pathogenesis. Curr.Top. Microbiol. Immunol. **180**:65-94.

O'Brien,A.D., and R.K.Holmes. (1996). Protein toxin of *Escherichia* coli and *Salmonella,*P2788- 2802. In F.C.Neidhardt, R. Curtiss *,J.L.Ingraham, E.C.C.Lin, K.B.Low, B. Magasanik, W.S.Reznikoff, M.Riley, M.Schaecher, and H.E.Umbarger (ed), Escherichia coli and *Salmonella*. cellular and Molecular Biology, 2nd ASM press, Washington D.C. In J.P.Naarto and J.B. Kaper. (1998). Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. **11**:142-201.

Oelschlaeger,T.A., T.J.Barrett, and D.J.Kopecko. (1994). Some structures and processes of human epithelial cells involved in uptake of enterohaemorrhagic *Escherichia coli* O157:H7 strains. Infect. Immun. **62**:5142- 5150.

Proulx,F., J.P.Turgeon, G.Delage, L.Lafeur, and L.Chincoine. (1992). Randomization, controlled trail of antibiotic therapy for *Escherichia coli* O157:H7 enteritis. J. Pediatr. **121**:299- 303.

Radostits.M.O, (2000). Textbook of Diseases of Cattle, Sheep, Pigs. 9th ed. Elsevier Health Science. P.784.

Reymond,D., R.P.Johnson, M.K.Karmali, M.Petric, M.Winkler, S.Johnson, K.Rahn, S.Renwick, J.Wilson, R.C.Clarkes and J.Spika. (1996). Neutralizing antibiotic to *Escherichia coli* verocytoxtoxic and antibobies to O157 lipopoly saccharide in healthy farm family member and urban residents. J. Clin. Microbiol. **34**:2053-2057.

Riley,L.W., R.S.Remis, S.D.Helgerson, H.B.McGee, J.G.Wells, B.R.Davis, R.J.Herbet, E.S.Olcott, M.Johnson, N.T.Hargrett, P.A.Blake, and M.L.Cohen. (1983). haemorrhagic colitis associated with a rare *Escherichia coli* serotype. N. Engl. J. Med. **308**:681- 685.

Ronald.M.A. (2006). Handbook of Microbiology Media for Examination of Food. 2nd ed. CRD press p.64-114.

Sack, R.B. (1981). Diagnosis of *Escherichia coli* infection. In: Acute enteric infection in children. Holme, Ti, Hdmgren, Merson, M.H., and Mollby, R.(ed). Elsevier/ worth Holland, Biomed. Press. p 444-459.

Saif.Y.M. (2003). Disease of Poultry. 11th ed Black well publisher p.631.

Samadpour, M., Liston, J. E. Ongerth, and P. I. Tarr. (1990). Evaluation of DNA probes for detection of Shiga-like-toxin-producing *Escherichia coli* in food and calf fecal samples. Appl. Envuron. Microbiol. **59**:1212-1215.

Samuel R, S.B.March, Rafiq.A, G.S.Bezanson, and S Kasatiya. (1988). Characterization of *Escherichia coli* Serotype O157:H7. J. Clin.Microbiol. **26**(10):2006-2012.

Schimdt, H., H.Karch,and L.Beutin. (1994). The large-sized Plasmids of enterohaemorrhagic *Escherichia coli* O157:H7 strains encode hemolysin which are presumably members of the *E. coli* α-hemolysin family. FEMS. Microbiol. Lett. **117**:189-196.

Schimdt, H., and H.Karch. (1996). Enterohemolytic phenotypes and genotypes of shiga-producing Escherichia coli O111 strains from patients with diarrhea and hemolytic uremic syndrome. J.Clin.Microbiol **34**: 2364-2367.

Scotland,S.M., H.R.Smith, G.A.Willshaw, and B.Rowe. (1983). Verocytotoxin production in strain of *Escherichia coli* determined by genes carried on bacteriophage. Lancet **ii**:216. In J.P.Nataro and J.B.Kaper. (1998). Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev.**11**:142-201.

Sherwood.L.Gorbach, Jonhn.G.Bartlett, Neil.R. (2004). Infectious Diseases. 3rd ed. Back well Lippincott Wiliams& Wilkin. p. 642.

Sears,C.L., and J.B.Kaper. (1996). Enteric bacterial toxins: mechanisms of action and linkage to intestinal secreation. Microbiol. Rev. **60**:167-215.

Smith,H.W., P.Green, and Z.Parsell. (1993). Vero cell toxins in *Escherichia coli* and related bacteria : transfer by phage and conjugation and toxic action in laboratory animals chicken and pigs. J. Gen. Microbiol. **129**:3121-3137.

Sussman. M. (1997).*Escherichia coli*: Mechanisms of Virulence. 8th ed Cambridge University press. p. 49.

Taylor, J., M.P.Wilkins, and J.M.Payne. (1991). Relation of rabbit gut reaction to enteropathogenic *Escherichia coli*. B.J.Exp.Pathol. **42**:43-52.

Thompson, J.S., D.S.Hodge, and A.A.Borczyk. (1990). Rapid biochemical tests to identify verotoxin-positive strains *Escherichia coli* serotype O157:H7. J. Clin. Microbiol. **28**:2165-2168.

Todar,K. (2002). Todar's online textbook of Bacteriology. University of Wisconsin-Madison, Department of Bacteriology. http://www.textbookofbacteriology.net.

Todar,K. (2005). Ken Todar's Microbial world. University of Wisconsin-Madison, Department of Bacteriology. http://www.textbookofbacteriology.net.

Torres,A.G., and S.M.Payne. (1997). Haem-iron transport system in enterohaeorrhagic *Escherichia coli* O157:H7. Mol. Microbiol. **23**:825-833.

Wallace,J.S,T.Cheasty,and K.Jones. (1997).Isolation of verocytotoxin-producing *Escherichia coli* O157:H7 from wild birds. J. Appl. Microbiol.**82**:399-404.

Wells.J.G, L.D.Shipman, K.D.Greene, E.G.Sowers, J.H.Green, D.N.Cameron, F.P.Downes, M.L.Martin, P.M.Griffin, S.M.Ostroff, M.E.Potter, R.V.Tauxe, and I.K.Wachsmuth. (1991). Isolation of Escherichia coli Serotype O157:H7 and other Shiga-Like-Toxin-Producing E. coli from Dairy Cattle. J. Clin. Microbiol. **29**(5)985-989.

Wells, J.G., B.R. Davis, I. K. Wachsmuth, L.W. Riley, R. S. Remis, R. Sokolow, and G. K. Morris. 1993. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare Escherichia coli serotype. J. Clin. Microbiol. **18**:512-520.

Wikipedia (2007). *http:enwikpedia.org/wiki/ Protobacteria.Enterobacteriacae.*

World Health Organization. (2005). Enterohaemorrhagic *Escherichia coli* O157:H7 (EHEC). http://www.who.int/emc.

Zhang, H.-Z., and M.S.Donnehberg. (1996). DsbA is required for stability of the type IV pilin of enteropathogenic *Escherichia coli*. Mol. Microbiol. **21**:787- 797.

Appendix

6-1 Culture media:-

6-1-1 Liquid media:-

6-1-1-1 Peptone water (Oxoid):-

Ingredients	gm/L
Peptone	10
Nacl	5

6-1-1-2 Glucose-phosphate medium (Barrow & Feltham, 1993):-

Ingredients	gm/L
Peptone	5
K2HPO4	5

6-1-1-3 Malonate Broth (Difco code NO 0395-01):-

Ingredients	gm/L
(NH4)2SO4	2.0
Yeast extract	1.0
K2HPO4	0.6
KH2PO4	0.4
Sodium malonate	3.0
Sodium chloride	2.0
Bromothymol blue, 0.2%aq.soln.	12.0 ml

6-1-1-4 Nutrient Broth (Biomark, code NO B274):-

Ingredient	gm\L
Peptic digest of animal tissue	5.00
Beef extract	1.50
Sodium chloride	5.00
Yeast extract	1.50

6-1-2 Semi- solid media:-

6-1-2-1 Hugh and Leifson's (OF) medium (Barrow & Feltham, 1993):

Ingredients	gm/L
Peptone	2.0
Nacl	2.0
K2HPO4	0.3
Agar	3.0
Bromthymol blue, 0.2%aq.sol	15.0

6-1-2-2 Motility medium (Barrow & Feltham, 1974):-

Ingredients	gm/L
Nutrient broth	13.0
Agar	1.1

6-1-3 Solid media:-

6-1-3-1 Nutrient agar (Hi Media code NO M001):-

Ingredients	gm/L
Peptone	5.0
Yeast extract	2.0
Lab Lemco powder	1.0
Sodium chloride	5.0
Agar No. 1	15.0

6-1-3-2 MacConkey′s agar (Hi Media code NO M081):

Ingredients	gm/L
Peptone	20
Nacl	5.0
Bile salts	1.5
Lactose	10.0
Agar	15.0
Neutral red	0.03
Crystal violet	0.001

6-1-3-3 Sorbitol MacConkey′s agar medium:

It was prepared according to.Harrigan (1998).

Ingredients	gm/L
Sorbitol	10.0
Peptone	20.0
Sodium desoxycholate	1.0
Sodium chloride	5.0
Neutral red (1.0% aqueous solution)	3.0
Crystal violate (0.1%aqueous solution)	1.0
Agar	15.0

6-1-3-4 Simmon's Citrate Agar (Difco code NO 0091-01):

Ingredients	gm/L
Ammonium dihydrogen phosphate	1.0
Magnesium sulphate	0.2
K2PO4	1.0
Sodium chloride	5.0
Sodium citrate	2.0
Bacto-Agar	15.0
Bacto-bromothymol blue	0.08

6-2 Reagents:-

6-2-1 Hydrogen peroxide:-

H2O2 3% aq.soln.

6-2-2 Oxidase test reagent:-

1% tetramethyl-p-phenylenediamine dihydrochloride aq.soln.

6-2-3 Methyl red solution:-

Contained:-

Methyl red	0.04g
Ethanol	40g
Distilled water to	100g

Prepared by dissolved methyl red ethanol and diluted with water.

6-2-4 α-naphthol solution:-

Contained α-naphthol in ethanol.

6-2-5 Kovacs reagent for indole test:-

Contained:-

P-dimethylaminobenzaldehyde	5g
Amyl alcohol	75g
Conc. HCL	25ml

The aldehyde was dissolved in alcohol by gently warming in water bath (about 50-55˚C). They were cooled and added to the acid. The reagent stored at 4˚Cand protected from light.

6-3 - Indicators:

6-3-1 Bromothymol blue (Hi Media laboratories).